Commonwealth Bureau of Animal Nutrition

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DIET IN RELATION TO REPRODUCTION AND THE VIABILITY OF THE YOUNG

PART I
RATS AND OTHER LABORATORY ANIMALS

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FOREWORD

THE earnings of animal husbandry are seriously reduced by losses due to inefficiency of the breeding stock and poor lactation or low vitality of the young. Fifteen years ago we reviewed in *Nutrition Abstracts and Reviews* the known causes and types of sterility in dairy cows, with the possibility in mind that diet might be implicated. The performance of dairy herds has not improved in the interval and that of other classes of stock is often little or no better. For that reason we planned to survey the position again and to extend the review to cover all the farm animals for which we might find worth-while information. It was obvious almost at once that it would be difficult to find records for significant numbers of very well fed animals, or even records of everyday performance along with records of diet, and that it would be still more difficult to find adequately controlled experiments of sufficient duration to make interpretation of the results reliable. We decided, therefore, to begin by reviewing the experiments on laboratory animals in the hope that there we might find results that would serve as a guide to the interpretation of experiments on farm animals, and because laboratory animals have become so important a part of scientific equipment that we believed many research workers would welcome the review.

This report, then, is concerned with estimates of the dietary requirements of laboratory animals for reproduction and lactation. Attention has been confined to diets used for stock colonies for which records of breeding performance have been kept, experimental modifications of such diets, and so-called " purified " or " synthetic " diets used to study the effect of single constituents of food. Experiments in which small animals have been used to assay the value of human diets or the diets of farm animals have been referred to here only when some finding, strictly relevant to the present subject, has emerged.

The literature abstracted in *Nutrition Abstracts and Reviews*, Volumes 1 (1931-32) to 17 (1947-48), has been covered, with reference to earlier papers where necessary. A few recent 1948 papers have been selected from those that appeared after the general compilation was complete, usually because they served to clarify some obscure issue. The volume of material discussed is large. The complications are many and sometimes tedious. Hence, whenever possible, the data have been summarised in tables and graphs. Paper has been economised by using chemical symbols for substances, which though incorrect is not really misleading.

The mere compiling of the data presented here has been an arduous task, but compilation alone serves no very useful purpose. Miss Russell set herself the much more difficult task of trying to show why, from such a large volume of work, the harvest of consistent findings should be so small. Reasons appear as the analysis develops, and we hope future workers may avoid some of the omissions and unnecessary complications that have made much of the work reviewed here so difficult to interpret.

Small animals have long been important tools of research for the study of drugs, or physiological processes, or infective disease and its control. Their importance for such purposes is not diminishing and they are now essential in every nutrition laboratory. The practice of having such test animals of standard genetic make-up is reasonably well established but it is not so well recognised that they should also be standardised in respect of dietary history. The reaction of test animals, whether to drug, galvanic battery, infective organism, or experimental diet, may be profoundly modified by diet ; and not only the diet on which the animal was reared but that of parents and grandparents. There is no shirking the fact

that much of the work done in nutrition laboratories has been bad and the results confused or contradictory because groups of animals used have differed in diet history. This report, therefore, in addition to the use we still hope to make of it to interpret experiments on farm animals may find a very practical application to secure a steady flow of well-born laboratory animals.

December, 1948 I. LEITCH.

ACKNOWLEDGEMENTS

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INTRODUCTION

In this review an attempt has been made to gather together published data showing the effects of diet on the ability of laboratory animals to produce and rear their young, and to assess the minimum daily intake of essential nutrients consistent with satisfactory reproductive performance. "Optimum" reproductive performance cannot be defined in terms of diet alone. The reproductive success obtainable under a given set of dietary conditions will depend on such factors as season of mating, environmental temperature, age and weight of females at first mating, maximum number of young given to the mother to rear and interval between matings, all of which have been shown to affect breeding performance (King, 1924; Mirskaia and Crew, 1930-31; Wan and Wu, 1931; Sorin, 1932; Barker and Ranson, 1932; Wishart and Hammond, 1933; Smith and Leverton, 1934a; Hall and Pierce, 1934; Enzmann and Crozier, 1935; Crozier and Enzmann, 1935; Hain, 1935; King, 1935; Rigdon, 1936; Smith *et al.*, 1938; Bohanan, 1939; Asdell *et al.*, 1941; Babcock *et al.*, 1940; Murray, 1941; Mehner, 1940; Brody, 1942; Bruce, 1947b).

The degree of selective breeding and of inbreeding practised are also important sources of variation. Alterations in litter size and sex ratio and in lactation performance have all been shown to result from selective breeding and inbreeding (King, 1918; Wright, 1922; Moore *et al.*, 1932; Falconer, 1947).

Since non-dietary factors, to which brief reference has been made above, are not standardised throughout reproduction studies, the reproductive performance obtained by one investigator cannot be used as a yardstick to measure quantitatively the reproductive success or failure obtained by dietary means in the experiments of other workers, nor indeed in other experiments of the same workers.

Rats

Table I shows the different results obtained with rats by one group of investigators using the same two diets (A and B) at different times (Sherman and Campbell, 1924, 1935, 1937; and Sherman *et al.*, 1945). It is, of course, possible that some of the variation in these results might be due to changes in the composition of the components of the diets, in this instance, whole wheat, whole milk powder and NaCl (see page 15). Nevertheless, misleading results may be obtained by attempting to correlate the findings of investigations in which the non-dietary factors affecting reproduction may not be strictly comparable.

TABLE I

A Comparison of the Average Reproductive Performance of Female Rats Obtained by One Group of Investigators at Different Times

Diet	Sherman Diet A			Sherman Diet B	
Year of Investigation	1924	1935	1937	1924	1945
No. of female rats studied	200	77	101	200	
Age of female at birth of first young (days)	155±1·5	137·3±2·7	132±2·4	112±1·1	
Duration of reproductive life of female (days)	186±7·7	184·9±9·24	213±9·4	322±8·9	331±10·6
No. of young born per female		20·7±1·10	24·1±1·13		
No. of young reared per female	5·8±0·5	9·2±0·70	12·7±0·74	18·1±0·8	29·6±1·21
Average weight of young at weaning (28 days)—males g.	34·0±0·5	37·2±0·09	38·9±0·08	40·3±0·2	43·3
females g.				39·2±0·2	42·6

Experiments to determine the effects of diet on reproduction in rats are of two main types: those in which the total reproductive performance of the female is recorded, and those in which only one reproductive cycle is studied. In both, the data may consist of the average results for several generations. Long-term investigations are preferable in the study of requirements, especially of nutrients of which mother and foetus normally build up reserves. Short-term studies may sometimes indicate the superiority of one diet over

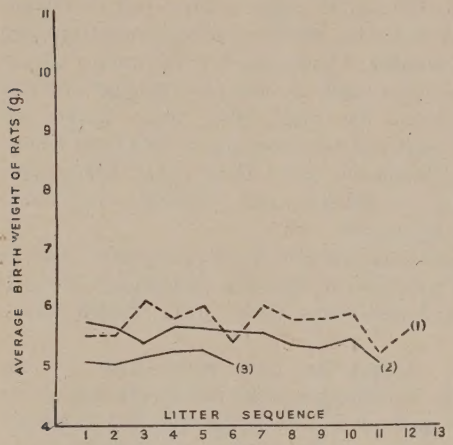

FIG. 1.—Birth Weight of Rats (Stock Diets).

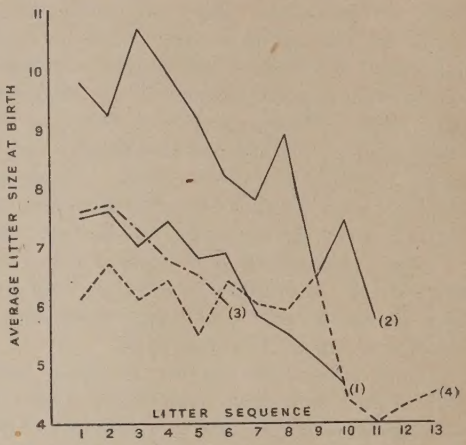

FIG. 2.—Litter Size of Rats (Stock Diets).

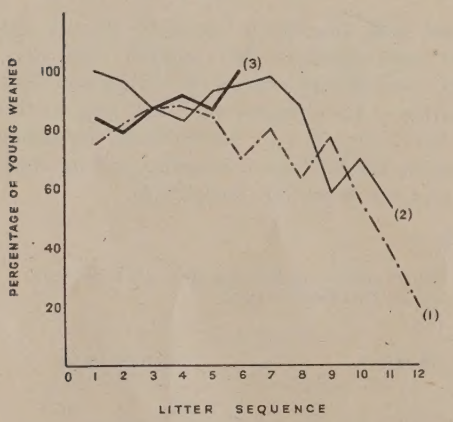

FIG. 3.—Percentage of Young Rats Weaned (Stock Diets).

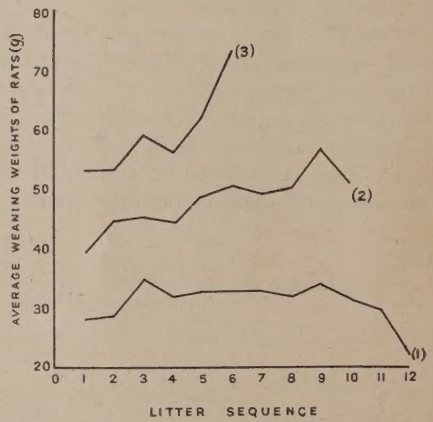

FIG. 4.—Weaning Weights of Rats (Stock Diets).

(1) Asdell *et al.* (1941). (2) Cox and Imboden (1936*b*). (3) Murray (1941). (4) King (1924).

another or the need for a particular nutrient in the diet. In such studies the numbers should be large enough to cover individual variation in response to the dietary essential under study and the question of parity should be taken into consideration: the results of first matings should be compared only with first, second with second (see figures 1 to 4).

All species

The dietary requirements for satisfactory reproduction in the female can be considered in three stages, the amounts necessary to enable the animal to show a regular oestrous cycle

and to become pregnant when mated with a fertile male, to allow successful gestation ending in the birth of a satisfactory number of live young, and to permit the rearing of a large proportion of the young to a good average weaning weight.

In attempting to assess the effects of dietary deficiencies on fertility in the female it is important that males of proved fertility should be used. For instance, vitamin E deficiency causes testicular atrophy and sterility in male rats, but does not prevent the occurrence of regular oestrous cycles and conceptions in female rats mated with fertile males.

Milk production is seldom measured directly in small animals. Instead, the liveweight increase of the litter is taken as an index of milk production and failure of the young to survive to weaning is frequently described as failure of lactation. In the same way, dietary constituents are described as essential for lactation because in their absence the young are not reared successfully. It is quite possible, however, where deficient diets have been fed to does from weaning or from the beginning of gestation that inability of the doe to rear her young may be due, not to inferior quantity or quality of the milk but, as in Mn deficiency in rats, to congenital debility of the young resulting from faulty nutrition *in utero*. For this reason " poor lactation " should be taken to mean inability to rear the young satisfactorily unless direct evidence is presented that the doe is incapable of suckling healthy stock young or unless milk production is measured directly (see also page 86).

Before proceeding to a review of the available experimental evidence indicating the nutrients essential and the amounts required for successful reproduction and rearing of young it may be of interest to give a brief account of stock diets which have given satisfactory results in the breeding of laboratory animals.

STOCK DIETS

Foods Used and Results Achieved

Considerable improvements in the breeding performance of rat colonies have been achieved by improvement in stock diets. This is illustrated by the following data of Mendel and Hubbell (1935), Table II, relating to the albino rat colony at the Connecticut Agricultural Experiment Station. Rats

TABLE II

Average Reproductive Performance of Connecticut Rat Colony under Different Dietary Conditions

Year	Fertile Matings per cent.	Young			
		Average Size of Litter at Birth	Percentage Weaned	Weaning Weight (at 21 days)	
				Male (g.)	Female (g.)
1912	86	7·2	71	23	26
Diet modified					
1919	65	6·3	67	31	31
1925	68	6·4	76	31	30
Diet changed 1931					
1935	93	9·6	90	48	47

Each female was permitted 3 matings with an interval of 3 weeks between weaning of a litter and re-mating. Litters were reduced to 8 at birth. The marked improvement in the

growth of the suckling young shown by the average for 1935 occurred immediately after the diet was changed in 1931, and was attributed to dietary improvement. No explanation is offered of the drop in fertility recorded in 1919 and 1925.

Smith *et al.* (1938) studied the breeding performance of rats of the Connecticut Agricultural Experiment Station colony over a period of 6 years. The diet consisted of a mixture of calf meal and cod liver oil to appetite, paste food to appetite for nursing mothers and young from weaning to 6 weeks of age, 3 g. wheat embryo weekly to each rat not supplied with paste food, 1 g. dried yeast daily and 10 g. lettuce weekly to each rat except nursing young. This diet, except for the inclusion of lettuce, was the same as that reported by Mendel and Hubbell (1935) as having been used since 1931. At the beginning of the experiment the percentage composition of the calf meal was linseed oil meal 15, ground malted barley 10, wheat red dog flour 22, dried skimmed milk 15, oat flour 15, yellow maizemeal 20, steamed bone meal 1, ground limestone 1, NaCl 1. This was given with cod liver oil in the proportion of 97 parts calf meal to 3 parts cod liver oil, as suggested by Maynard (1930). In the course of the experiment 1·5 per cent. of soluble bloodmeal was substituted for an equal amount of dried skimmed milk, the limestone was replaced by 1 per cent. of yellow maizemeal, and 0·25 per cent. of cod liver oil reinforced with a vitamin A and D concentrate was incorporated in the meal. On the basis of this final change a mixture of 98 per cent. of calf meal and 2 per cent. of cod liver oil was subsequently given. The paste food consisted of casein 25, whole milk powder 25, wheat embryo 20 and lard 30 per cent. The protein content of the dried calf meal was 22·1 per cent. and of the dried paste food 34·9 per cent. Smith *et al.* (1938) considered, on the basis of current knowledge, that this diet provided a generous allowance of dietary constituents known to be required for satisfactory reproduction and lactation and for growth of the young. Females on this diet were first bred at 110 to 120 days of age. Litters were reduced to 8 at birth and weaned at 21 days. When the interval between weaning a litter and re-mating was 2 weeks the following average results were obtained for a total of 4 matings in each of 7 generations: percentage fertility 91·8; average number born per litter 9·4; average percentage weaned per reduced litter 83·2; weaning weights of the young, males 43 g., females 42 g.

Kellermann (1934) described a stock ration for breeding albino rats used with excellent results at Onderstepoort for over a year. The ration consisted of ground yellow maize 68, linseed oil meal 10, crude casein 5, dried brewer's yeast 5, alfalfa meal 3, butterfat 5, dried beef liver 2, bone ash 1, $CaCO_3$ 0·5 and NaCl 0·5 per cent. This dry comminuted ration contained 4·5 per cent. of crude fibre and had a protein content, excluding the N of yeast, of 15·69 per cent. The distribution of food energy when expressed in terms of percentages of total energy (including the calories derived from yeast) was 68·2 from carbohydrate, 15·0 from fat and 16·8 from protein. The ash, Ca, P and Mg contents were 3·72, 0·61, 0·44 and 0·16 per cent., respectively, the Ca : P ratio being 1·39. The I content of the ration was 33 μg. per 100 g. The dry ration was supplemented daily by fresh whole milk to appetite. Litters were reduced to 6 at birth, and 90 per cent. of the litters produced in the course of over a year weighed 40 to 60 g. at an age of 23 days. A similar ration was used by Murray (1941) for breeding stock and for young growing albino rats at Onderstepoort over a period of 4 years, during which the rats remained healthy. This consisted of a meal ration containing yellow maizemeal 75, linseed oil meal 15·5, lucerne meal 5, crude casein 2·5, bone meal 1, $CaCO_3$ 0·5 and NaCl 0·5 per cent., supplemented daily by fresh milk. The protein content of the meal mixture was 15 per cent. The reproduction records of rats on this diet are shown graphically in figures 1 to 4. In food consumption studies Murray used a meal mixture containing yellow maizemeal 65, linseed meal 15, crude casein 7, yeast 3, lucerne meal 2, bone meal 2,

cod liver oil 2, $CaCo_3$ 0·5 and NaCl 0·5 per cent., with a protein content of 18·5 and a crude fibre content of 3·0 per cent. In this study, the results of which are presented graphically in figure 5, the meal was the only food given.

The stock diet used at the Rowett Research Institute has been described by Thomson (1936). A mixture of wheat offal (fine middlings No. 2) 19·2, ground wheat 19·2, Sussex ground oats 19·2, ground barley 9·5, ground maize 9·5, meat and bone meal (45 per cent. protein) 9·5, dried skimmed milk 7·0, white fishmeal (60 per cent. protein) 4·7, dried yeast (40 per cent. protein) 1·2, NaCl 0·5, and cod liver oil 0·5 per cent., is made into cubes. The Ca and P contents of this mixture are 1·18 and 0·99 per cent., respectively, giving a Ca : P ratio of 1·19. This meal mixture is supplemented by green food and fresh whole milk. In 1936 Thomson reported that this diet had been used successfully for $3\frac{1}{2}$ years, during which time over 6,000 rats had been bred and reared. Litters were reduced to 8 at birth and the average weaning weights of males and females at 21 to 23 days were 43 g. and 41·5 g. respectively, for both albino and hooded stock. This ration was modified during the war : the meal mixture now contains 14 per cent. of dried skimmed milk, the remaining constituents other than yeast being proportionately reduced. Fine bran has replaced the middlings as wheat offal because middlings were unobtainable during the war, and fresh whole milk is given to nursing females only, at the rate of 20 ml. per head daily (private communication, 1948). The composition of the new cubes as given by the manufacturer in 1948 is as follows : wheat offal (bran) 17·7, ground wheat 17·7, Sussex ground oats 17·7, ground maize 8·8, ground barley 8·8, white fish meal (60 per cent. protein) 4·5, meat and bone meal (45 per cent. protein) 8·8, dried skimmed milk 14·0, dried yeast 1·2, NaCl 0·4 and cod liver oil 0·4 per cent.

Wiesner and Sheard (1935) reported a mortality rate of 30·6 per cent. in young rats before the age of 30 days in a sample of 250 litters chosen at random from records of over 5,000 stock litters of the albino rat colony at the Institute of Animal Genetics, Edinburgh, but unfortunately the quantities of the various foods in the diet are not given.

The stock diet used by Cox and Imboden (1936*b*) in reproduction studies with rats of an inbred Wisconsin strain, the results of which are shown graphically in figures 1 to 4, consisted of yellow maize 57, whole milk powder 25, linseed oil meal 12, crude casein 3·7, alfalfa leaf meal 1·5, iodised table salt 0·4 and $CaCO_3$ 0·4 per cent. These workers found that a purified diet consisting of casein (washed with dilute acetic acid) 20, dextrin 55·1, lard 9, dried protein-free aqueous extract of brewer's yeast 4, wheat germ oil 1.6, carotene solution in maize oil

TABLE III

REPRODUCTIVE PERFORMANCE (AVERAGE OF 3 GESTATIONS) OF RATS ON STOCK AND PURIFIED DIETS

	No. of Females	Young				Weight Gain of Doe during Cycle (g.)
		Average Litter Size	Average No. in Litter Allowed to Suckle	Per cent. Raised	Average Weight at 21 Days (g.)	
Stock diet	10	9·9	5·9	94·4	42·9	34·9
Purified diet—						
1st generation	5	10·0	5·9	100	42·7	33·6
2nd ,,	9	9·9	6·0	93·2	43·2	32·3
3rd ,,	19	8·8	5·5	86·2	42·9	28·6

0·3, Ca acetate 1, salt mixture 3·1, and rice cellulose 5·9 per cent. gave equally good growth, reproduction and lactation in rats when used as the stock diet. The salt mixture consisted of NaCl 24·50, $MgSO_4$ 9·14, $KHCO_3$ 59·20, KCl 3·84, Fe citrate 2·94, $CuSO_4$ 0·32, $MnSO_4$ 0·04 and KI 0·02 per cent. The reproduction records shown in Table III represent the average performance in 3 successive gestations of rats on the stock diet and on the purified diet (Cox and Imboden, 1936*c*).

Reference has been made already to the stock diet (A) used by Sherman and his associates at Columbia University (see Table I for reproductive performance of rats). This diet consisted of 1 part dried whole milk and 5 parts ground whole wheat with NaCl added at a level of 2 per cent. of the weight of wheat. Campbell and Sherman (1945) reported that rat families in their colony were thriving in the 59th generation on this diet and reported improvement in breeding performance when the proportion of milk powder was increased to 1 part to 2 parts of ground whole wheat. This diet (Sherman B) had an average protein content of 16·0 per cent., Ca 0·34 and P 0·5 per cent., with an energy value of 4 Cal. per g. The average reproductive performance of albino rats on this diet is shown in Table I.

The stock ration used in the Food and Drugs Laboratory of the Canadian Department of National Health and Welfare in Ottawa (private communication, 1947) consists of commercially prepared cubes containing animal liver meal, a riboflavin supplement, skimmed milk powder, dried brewer's yeast, wheat germ, dehydrated alfalfa meal, dried meat, fish meal, soya bean oil meal, cooked cornflakes, cooked wheat flakes, dried beet pulp, bone meal, iron sulphate, bran, KI, ground limestone, $\frac{1}{2}$ to 1 per cent. of iodised salt, manganese sulphate and a fish liver oil fortified with vitamins A and D. The ration is made up to provide minima of 20 per cent. of crude protein and 3·5 per cent. of crude fat and a maximum of 5 per cent. of fibre. Nursing mothers receive fluid milk daily in drinking bottles. Breeding records of the rat colony on this diet are shown in Table IV.

TABLE IV

Breeding Records of Rat Colony, Food and Drugs Laboratory, Ottawa

Year	Average No. Young Born per Litter	Per cent. Stillbirths of Rats Born	Average No. Young Raised per Litter	Per cent. Raised
1942	8·4	0·99	5·99	71·3
1943	8·4	1·19	6·36	75·7
1944	8·8	1·55	6·06	74·1
1945	8·9	1·92	6·09	77·04
1946	8·1	2·08	6·04	78·02

These cubes, supplemented by small amounts of green or yellow vegetable in season and additional cod liver oil on bread once a week, are used also at the Animal Diseases Research Institute in Quebec. In this colony stillbirths are reported to be rare and infant mortality rate extremely low (private communication).

Nelson and Evans (1947*a*) have reported reproductive performance (Table V) of rats of the Long-Evans strain on a stock ration consisting of ground whole wheat 67·5, casein 15, whole milk powder 10, crisco 5·25, $CaCO_3$ 1·5, NaCl 0·75 per cent., with 3·5 g. fish oil concentrate containing 3,000 I.U. vitamin A and 400 chick units vitamin D per g., added to each kg. of diet. The approximate composition of this diet is given as protein 24·3, carbohydrate 54·8 and fat 11·0 per cent.

TABLE V

AVERAGE BREEDING PERFORMANCE OF RAT COLONY IN 1938 (NELSON AND EVANS, 1947a)

No. Rats Bred	Young					
	No. Litters	Total No. Born	Per cent. Born Dead	Average Weight at 1 Day (g.)	Average Weight at 21 Days (g.)	Per cent. Weaned at 21 Days
100	100	880	2	6·0	49 (male) 48 (female)	89–93

Mice

The National Institute of Nutrition in Buenos Aires has reared its rat colony since 1938 (12 generations, 1700 litters) on a diet of refuse from the Institute canteen to which is added 50 g. bone meal, 2 litres milk and 1 kg. raw meat per 18 kg. other foods. A careful study was made in 1943-44 of the food consumed and the rate of growth of rats of both sexes and all ages, and the diet was analysed daily for 17 days (Escudero *et al.*, 1947). No information is given about the usual size of litters or number reared in the colony. The average weights at birth and 21 days are given as: males 5·31 g. and 44·22 g., females 4·75 g. and 44·69 g.

Watson (1937) studied the reproductive performance of a group of 50 female and 6 male black-and-white mice on a stock ration of coarse oatmeal 92, cod liver oil 1, yeast extract 2 and wheat bran 5 per cent., with a calculated protein content of 11·87 per cent. In addition each mouse was given daily approximately 2 ml. of a mixture of equal parts milk and water. Comparable groups of mice were given the stock diet modified in a number of ways. In an experimental period of 26 weeks from the first day of mating 12·5 per cent. of does on the stock diet did not produce litters. The proportion due to resorptions and to actual sterility was not determined. In the groups of does given a diet in which the oatmeal was reduced from 92 per cent. to 33 or 40 per cent. and dried separated milk 25 or 27·5 per cent., coconut oil 4 or 4·5 per cent. and dextrin or a flour and water biscuit added to make up the remaining 92 per cent. of the ration, the corresponding percentages of does which did not produce litters were 3·5 to 4·0. The percentage of young alive at the age of 4 weeks was approximately 90 on the improved rations compared with an average of 66 on the stock ration. The calculated protein contents of the improved rations were 14·7, 16·7 and 17·4 per cent.

Schneider and Webster (1945) studied reproduction in W-Swiss mice on the Sherman B rat diet described above. The life cycle performance of 4 generations is shown in Table VI.

TABLE VI

REPRODUCTION AND REARING OF YOUNG BY MICE ON SHERMAN B DIET

Generation	No. Females Bred	No. First Litters Born within 30 Days	Young			
			Average Size Litter at Birth	Total No. Born	Per cent. Weaned	Av. Weight at Weaning (g.)
1	20	20	8·2	164	70·1	9·7
2	15	15	6·46	97	83·5	9·3
3	15	15	8.00	120	66·7	9·1
4	14	13	7·38	96	84·4	9·0

When maize, rye, rice, potatoes and oats were tested as substitutes for wheat in the diet reproductive performance was poorer. The substitutes are listed in approximately descending order of merit.

The stock diet used for the mice breeding colony at Onderstepoort consists of commercially manufactured cubes of the following percentage composition: yellow maizemeal 47·35, peanut cake meal 7·20, linseed oil cake meal 2·33, meat and bone meal 9·37, white fish meal 2·33, Sussex oats 14·00, wheaten bran 7·20, lucerne meal 0·95, cod liver oil 0·10, brewer's yeast 0.95, cane molasses 7.20, NaCl 0·47 and $CaCO_3$ 0·47. These cubes contain protein 19·16, fat 5·44, fibre 3·19, ash 6·81, moisture 10·70, N-free extract 54·70 per cent. The Ca and P content is 1.47 and 0.93 per cent. respectively. The cubes contain per lb. 1,446 I.U. vitamin A and 6·69 mg. vitamin E, and are estimated to supply vitamin B_1 2·1, riboflavin 1·35, nicotinic acid 21·75, pantothenic acid 6·9 and choline chloride 388·0 mg. per lb. A small quantity of green feed is given once a week.

On this diet a large number of mice have been produced for experimental purposes and birthweight, litter size and growth are described as normal (private communication, 1947).

Rabbits

Bruce (1947*a*) reported good reproduction in rabbits of the Dutch variety on a diet of bran 15, barley meal 20, groundnut meal 15, linseed cake 10, meat and bone meal 8, dried grass meal 30, $CaCO_3$ 1 and NaCl 1 per cent. The diet was calculated to contain crude digestible protein 16·5, soluble carbohydrate 33·7, fat 4·6 and fibre 6·7 per cent. and provided from 2 to 6 mg. ascorbic acid per 100 g. depending upon the quality of dried grass used. Hay and unlimited water were given also, but no fresh green feed. The results obtained are shown in Table VII.

TABLE VII

Reproduction in Rabbits on Stock Diet

Group	Males	Females	No. Females	No. Litters Born	Av. Size of Litter at Birth	Per cent. of Young Weaned at 35 Days
1	Crossbred	Dutch	9	9	6·44	74
2	Dutch	Dutch	9	7	6·00	67
3	Offspring of Group 2		11	11	6·18	93

Practical rations for breeding rabbits are given in a previous Technical Communication from this Bureau (Wilson and McCartney, 1940).

Syrian hamsters

Hamilton and Hogan (1944) described a stock diet on which female hamsters reared 5 litters without showing evidence of nutritional deficiency. The diet consisted of ground wheat 28, wheat germ 7, alfalfa meal 5, dried skimmed milk 15, linseed oil meal 12, ground maize 20, dried yeast 10, $CaCO_3$ 1, NaCl 1 and vitamin A and D concentrate 1 per cent. The concentrate supplied 1,200 I.U. vitamin A and 170 I.U. vitamin D per g. Breeding records are shown in Table VIII.

TABLE VIII

Reproduction in Syrian Hamsters on Stock Diet

Litter Sequence	No. of Litters	Average No. Young Born per Litter	Per cent. Weaned	Average Weight at Weaning (g.)
1	10	4·4	52	25·0
2	10	6·2	52	29·0
3	9	6·1	60	26·7
4	6	4·7	61	28·0
5	3	3·3	90	24·6

Discrepancies Due to Variations in Composition of Ingredients of Diets

It should be emphasised that diets made in different laboratories from the same recipe may give different results. This was the experience of Coward *et al.* (1932) who found that a diet which had been in use in Wisconsin University laboratories for about 9 years and the Sherman B diet, when made up in their laboratory in Britain, were unsatisfactory for breeding rats. The Sherman B diet of whole wheat, whole milk powder and NaCl has been prepared recently from ingredients available at the Rowett Research Institute in Britain and found to contain only 14·4 to 14·7 per cent. of protein (private communication, 1948), compared with the average of 16 per cent. reported by Sherman and his colleagues. Differences in the protein content of wheat from different sources are well known and other differences, such as in vitamin B_1 content, have been reported. Similarly differences in composition of other dietary ingredients may be expected to occur which may or may not be important in determining reproductive performance on the diet.

FOOD CONSUMPTION AND ENERGY REQUIREMENTS

Habitual Food Consumption and Energy Equivalents

OESTROUS CYCLE

Rats

In a recent study of energy exchange in female rats as affected by the oestrous cycle Brobeck *et al.* (1947) found that under carefully controlled environmental conditions, including constant environmental temperature, the food intake, bodyweight and body temperature were all lower during oestrus than during di-oestrus. Food intake during di-oestrus often exceeded intake during oestrus by as much as 6 g. per day, the equivalent of 18 Cal. where the average total energy intake was 35 to 45 Cal. Associated with changes in food intake were variations in bodyweight amounting to as much as 10 to 12 g. during individual cycles. These data are in agreement with the earlier observation of Slonaker (1925) that the food intake of rats was lower during oestrus than during di-oestrus.

PREGNANCY

Murray (1941) found that the food consumption of pregnant rats on a stock meal mixture containing 18·5 per cent. of protein averaged 13·4 g. per day in the first week of pregnancy, 15·5 g. in the second week, 16·9 g. in the third week and 15·3 g. for the whole period. This represents an increase of 26 per cent. from the first to the third week after service. According to Slonaker (1927), the daily food intake increased by 2 g. during pregnancy, but his non-pregnant female albino rats ate about 19 g. daily compared with an average of 12·5 g. in Murray's experiments. Murray suggests that this difference was probably due to the fact that Slonaker's rats were in revolving cages and were therefore expending more energy.

Cole and Hart (1938) observed an increase of appetite in rats 48 hours after copulation, and the average food consumption during pregnancy was 30 per cent. greater than in non-pregnant controls. Unlike Murray, they found that food consumption remained almost constant throughout pregnancy.

LACTATION.

Murray (1941) found that during a lactation period of 28 days, female rats with 5 and 7 young ate only 9 and 14 per cent. more food than does with 4 young. Larger litters caused much greater differences in food intake. The average daily food consumption of female rats with litters of 7 or less was 31 g. and of those with litters of 8 or more was 42 g. These

results are in good agreement with those of Slonaker (1925), who recorded an average food consumption during 25 days of lactation of 27·3 g. per day for female albino rats with litters of 6 or less, and 38·6 g. for those with litters of 7 or more. Murray's over-all average daily food intake for rats during the lactation period was 36 g. This is close to the value obtained by Slonaker (1925) for the first 25 days of lactation, and by Hitchcock (1927) for rats receiving a basal diet of wheat flour, casein, skimmed milk powder, cottonseed oil and salts supplemented by meat to appetite. Another group of rats on this diet without meat consumed on the average 30 g. daily. In both groups the average litter size was 7 and the lactation period was 30 days. Nelson and Evans (1947*c*) reported an average food intake during a lactation period of 21 days of 36·3 g. per day by rats of the Long-Evans strain on a stock diet. The litters were reduced to 6 at birth.

For a group of rats continuously re-mated after weaning of a litter throughout the reproductive span, with an average litter size of 7, Slonaker (1927) recorded a daily food consumption of 30 g. during the first 20 days of lactation. The variations in food consumption during gestation and lactation as observed by Slonaker (1925) and Murray (1941) are shown below (figure 5).

FIG. 5.—Food Consumption during Gestation and Lactation in Rats (Stock Diets).

Slonaker (1927) estimated that in his group of rats, with an average litter size of 7, in all 78 Cal. were required over maintenance for each pup during the 22 days of gestation, and 279 Cal. during the 22 days of lactation. In computing the requirements during lactation allowance was made for the number of young which died and were, therefore, not nursed for the whole period.

Hitchcock (1927) estimated the calories available for milk production after allowance had been made for maintenance. From his data it can be calculated that in the group with

an average food intake of 30 g. daily, the young to 22 days of age required approximately 234 Cal. per head. This estimate is based on the assumption that all the young were reared, so the actual value per head should probably be higher. There is, therefore, good agreement between these two estimates.

In terms of food, Slonaker's figures represent a requirement of 20 g. food per rat pup during the 22 days of gestation, and 71 g. per pup during the 22 days of lactation. Murray estimated that the amount of food required during the lactation period of 28 days for each additional rat pup from 4 to 11 was 72 g.

Hitchcock (1927) estimated that in groups of rats on a diet containing meat and on a meatless diet the food consumed by the mothers during 30 days of lactation was equivalent, respectively, to 10·6 and 11·5 Cal. per g. increase in weight of the young. The former group reached an average weight of 61·8 g. and the latter of 47·3 g. at 30 days of age.

Restricted Food Intake

Rats, Guinea-pigs

Over a long period evidence has accumulated which shows that retardation of growth of rats and guineapigs caused by underfeeding delays the onset of sexual maturity and may cause cessation of oestrus and ovarian atrophy in the female and testicular degeneration in the male. Resumption of normal oestrous cycles and reversal of the process of degeneration in the male can be effected by re-feeding (Loeb, 1917; Papanicolaou and Stockard, 1920; Evans and Bishop, 1922; Marrian and Parkes, 1930; Macomber, 1933; Mason, 1933; Bortulucci and Zambonini, 1934; Asdell and Crowell, 1935; Conte-Marotta, 1937; Werner, 1939; Stephens and Allen, 1940; Mulinos and Pomerantz, 1941; McCay *et al.*, 1943; Drill and Burrill, 1944).

Sexual maturity

Rats

Recently Scheer, Soule *et al.* (1947) showed that when rats were restricted to an energy intake of 12 Cal. per day from a stock diet or from experimental diets of different fat contents for a period of 12 weeks from weaning none of the females matured during the period of restriction. The vagina opened after 1 to 2 weeks of re-feeding depending on the type of diet. After 5 to 6 weeks of feeding to appetite female rats were mated with males of their own group. The percentage of successful matings ranged from 60 to 100. Some of the females which failed to mate with male rats from the same group were mated with stock male rats and all of them produced litters. The ability of the female rats to rear their young was not inferior to that of litter mates whose diets had not been restricted (Deuel *et al.*, 1947). In another experiment (Scheer, Codie *et al.*, 1947) rats were maintained on stock diet for 19 weeks from weaning and then given the experimental diets at a level of 24 Cal. per head daily for 8 weeks, 12 Cal. per head per day for the next 4 weeks and subsequently to appetite. From the beginning of the re-feeding period male and female rats from the same group were caged together but few litters were produced and none was successfully reared to weaning despite the small size of the litters. The fertility of the females was not checked by using stock males in this experiment.

Oestrous cycle.

Mice

It has been shown by Ball and her associates that a restricted intake of calories by female mice whose intake of protein, vitamins and minerals was approximately the same as for normal controls, resulted in decreased frequency of oestrous cycles, infantile uteri and failure of growth of mammary glands (Ball *et al.*, 1943; Huseby *et al.*, 1945). White *et al.* (1944) also have observed inhibition of oestrus in mice resulting from calorie restriction.

FERTILITY.

Ball *et al.* (1947) restricted the calorie intake of 72 weanling female mice to approximately three-quarters that of control litter sisters fed to appetite, the intake of protein, minerals and vitamins being the same in both groups.

The females on the restricted intake were mated daily from 4 months of age with male mice of proved virility. When a female became pregnant her litter mate control was mated. At the age of 240 days 26 of the mice whose food intake had been restricted were given the control diet to appetite while the diet of the others continued to be restricted. The fertility indices, *i.e.*, the average numbers of fertile matings per mouse per month for each group, were compared. Between the age of 243 and 374 days the fertility indices for the restricted and previously restricted groups, respectively, were 0·04 and 0·65 ; the controls had fallen to 0·05 at this age. The fertility index of 0·65 for females given food *ad libitum* after calorie restriction to 8 months of age is approximately the normal for young full fed females. Fertility was therefore not impaired by the delayed maturation.

Although Ball *et al.* (1947) had ensured a normal protein intake during the period of restriction they point out that whenever the non-protein calories are insufficient to meet basal and activity energy requirements protein will be burned as fuel and the picture may be complicated by deficiency of amino-acids.

PREGNANCY.

Rats

The effect of restricted food intake during pregnancy was studied in rats by Barry (1920). According to a review by Warkany (1945), Barry found that in albino rats starvation instituted shortly after copulation resulted in resorption. Starvation from the 11th day of gestation did not interrupt pregnancy ; in the majority of cases gestation was prolonged. There were few resorptions and no premature delivery. About one-third of the offspring were stillborn and the average number of young per litter was low. The average weight of the young born of mothers starved during the latter half of pregnancy was 40 per cent. below the normal birthweight.

Rabbits

Jonen (1931) found that in rabbits, starvation begun during the first few days ofpregnancy resulted in death of the embryos. If fasting began in the second half of pregnancy, premature delivery occurred 10 to 12 days later. The foetuses were smaller and lighter than those of control mothers.

Impaired Appetite

Most dietary deficiencies cause anorexia and the extent to which the observed effects of such deficiencies on the oestrous cycle, reproduction and lactation of laboratory animals are primary or secondary to reduced food intake should be determined by means of paired feeding experiments.

PROTEIN

The importance of individual amino-acids in the dietary protein for growing animals has been demonstrated by a large body of experimental work, mainly with rats. Some attempts have been made also to determine the importance of a few of these amino-acids for reproduction and lactation and the quantity and type of protein required to supply the needs of breeding stock.

Restricted Supply and Restricted Sources of Protein

OESTROUS CYCLE

Rats

Guilbert and Goss (1932) showed that in rats which had normal oestrous cycles on diets containing 7 to 17·5 per cent. of protein, oestrous cycles became irregular and usually ceased when the protein content was reduced to between 3·5 and 4·0 per cent. by replacement of part or all of the foods containing animal protein by maize starch. Although they found that 7 per cent. of protein of mixed origin was adequate for normal ovarian function, it has been shown that as much as 18 per cent. of protein in the diet may be inadequate when the sole source of protein is wheat gliadin (Courrier and Raynaud, 1932; Pearson, 1936). Inclusion of 0·6 per cent. *d*-lysine in place of that amount of gliadin restored the oestrous cycle to normal (Pearson, 1937). Pearson *et al.* (1937) showed that a diet containing 5 per cent. of casein as sole source of protein inhibited oestrus in the rat. Addition of cystine did not restore oestrus, but when 5 per cent. gliadin was included also normal sexual rhythm was restored. A diet containing 10 per cent. of casein as sole source of protein had no adverse effect on the oestrous cycle (Pearson, 1936). A diet containing 10 per cent. of gliadin with added lysine was also adequate for normal oestrus (Lafon and Veillet, 1938).

In all the above investigations the presence or absence of normal oestrus was determined by the vaginal smear technique, but recently Freed *et al.* (1939) have cast doubt on the adequacy of this method of detecting ovarian dysfunction. They found that normal rats on a low-protein diet (7 per cent. of protein) developed vaginal oestrus in the presence of uterine di oestrus and ovarian dysfunction. Guilbert and Goss (1932) found that when rats which showed vaginal oestrus on low levels of protein were subjected to breeding tests with normal male rats, litters were not produced. This was due to failure to mate, infertile matings or fertile matings followed by death of the embryo.

Slonaker (1931*b*) found that when female rats on a diet containing 10 per cent. of protein (mixed animal and vegetable) were mated with normal male rats of proved fertility, 96 per cent. of the matings were fertile. This is in general agreement with the finding of Pearson *et al.* (1937) and of Lafon and Veillet (1938), that a 10 per cent. level of suitable protein in the diet is adequate for the normal sexual rhythm of female rats.

Aschkenasy Lelu and Aschkenasy (1947) studied the oestrous cycle of adult female rats after 4 months on a diet containing from 0 to 90 per cent. of casein. The diet contained extract of malt, oil 5, salt mixture 3, and dried brewer's yeast 1·5 per cent. with casein 0 to 90 per cent. and starch to 100. Vitamin E was given. When the diet contained 0 or 2 per cent. of casein di-oestrus was prolonged, when it contained 50, 80 or 90 per cent. of casein oestrus was prolonged. At levels of 7, 15 and 30 per cent. the oestrous cycle was normal.

White and Andervont (1943) observed that in the mouse a diet low in cystine produced anoestrus. Deficiency of lysine had the same effect (White and White, 1944). Mice

Venzkovsky (1930) observed that in female rabbits on a protein-free diet pregnancy did not occur after mating. This was true even if the rabbits were normally fed until after copulation. The sterility was attributed to atrophy of the uterus and ovaries. Rabbits

On the other hand, Friedman and Turner (1939) state that in rabbits restriction to a protein-free diet or a diet containing only 2 per cent. of protein for relatively long periods has very little effect upon oestrus and ovulation, despite the fact that the animals lose about 25 per cent. of their initial bodyweight.

TESTICULAR DEVELOPMENT AND FUNCTION

Rats

Courrier and Raynaud (1932) found that when male rats were given a diet containing 18 per cent. of gliadin as sole source of protein, and hence deficient in lysine, the testicles were undersized. The diet consisted of gliadin, starch, butterfat, lard and salts, supplemented with wheat germ, malt flour, lemon juice, cod liver oil and hemp seed oil.

In young male rats kept for 210 days on a diet containing 6 per cent. of casein as source of protein, Lafon (1937) observed retardation of testicular growth but no loss of fertility as compared with rats receiving a supplement of cystine.

Maun *et al.* (1945) found that young rats given a synthetic diet deficient in phenylalanine for 28 days, although they consumed as much food as the pair-fed controls with a supplement of phenylalanine, lost weight more rapidly, and the male rats showed marked testicular atrophy and failure of sperm formation.

FERTILITY

Rats

Some workers have claimed to show that lack of tryptophan in the diet of male and female rats adversely affected fertility and reproduction (Albanese *et al.*, 1943 ; Keller, 1946) but the diets used were obviously deficient in other nutrients known to be essential. Berg and Rohse (1947) using a diet lacking only tryptophan failed to confirm the findings of Keller.

PREGNANCY

Rats

Seegers (1937) showed that pregnant albino rats bore young although given a diet free from N for 13 days before term. Such a diet given prior to the 8th day before delivery resulted in undernourishment of the foetus. The young weighed less at birth and contained less N, total and percentage, than those of adequately fed stock rats.

Rabbits

Venzkovsky (1939) found that a protein-free diet given to pregnant rabbits caused abortion or foetal death attributable to lesions of the placenta.

Comparison of Different Levels of Protein in the Diet

Records of Performance

Rats

The most extensive study of the effect of different levels of protein in the diet on the reproductive performance of the albino rat is that of Slonaker (1931 *a*, *b*, *c*, *d* ; 1938). Rats were fed from weaning on a basal diet of maize starch 48·3, whole ground wheat 19·3, whole ground maize 9·7, skimmed milk powder 3·9, ground alfalfa leaves 3·9, commercial casein 1·9, meat scrap 1·0, wheat germ 2·9, unsalted butter 4·8, yeast 1·9, NaCl 1·0 and $CaCO_3$ 1·4 per cent. In this ration 10·3 per cent. of the energy was in the form of protein. Diets in which 14·2, 18·2, 22·2 and 26·3 per cent. of the energy came from protein were obtained by adding meat scrap to this basal diet. Each diet had an energy value of 3·82 Cal. per g. from which it can be calculated that the protein contents of the diets were 9·8, 13·5, 17·4, 21·2 and 25·1 per cent.

It should be noted that Slonaker's figures for percentage of calories from protein are usually quoted as percentage of protein in the diet. In his diets and in the type of diet usually fed to rats the two are almost equal, but with diets containing fresh foods, such as vegetables, the protein must be stated either in terms of percentage of calories from protein or as percentage in the dry food, if the results are to be compared with those for dry diets.

The combined results for 6 generations of rats are summarised in Table IX.

TABLE IX

AVERAGE REPRODUCTIVE PERFORMANCE OF 6 GENERATIONS OF RATS ON DIETS CONTAINING DIFFERENT LEVELS OF PROTEIN

Percentage of Calories from Protein	10	14	18	22	26
Percentage of sterile males	5·25	2·70	22·89	40·00	33·77
Percentage of sterile females	6·40	2·59	23·00	38·56	21·69
Age of females at opening of vagina (days)	68·7	55·2	51·1	57·8	56·8
Average interval between mating of females and birth of first litter (days)	65	67	91	131	84
Reproductive span of females (days) (beginning of first gestation to end of last lactation)	271	289	257	184	265
Total breeding performance of females					
Number of mothers	94	102	84	46	74
Number of young produced per mother	23·3	20·8	17·4	13·8	18·6
Average number of litters	4·78	4·43	3·89	2·93	3·41
Average size of litter	4·82	4·73	4·43	4·71	5·48
Average birthweight of young males, g.	5·15	5·22	5·37	5·29	5·19
females, g.	4·97	4·98	5·14	4·98	4·98
Lactation					
Number of litters nursed	212	230	192	64	146
Average percentage loss of weight in mother	7·82	8·03	5·82	3·73	6·49
Average loss of weight per young nursed, g.	3·10	3·62	2·80	1·69	2·67
Average size of litter nursed	4·6	4·70	4·46	4·69	5·31
Average weight at 25 days—males, g.	20·8	26·7	30·8	28·4	32·5
females, g.	20·2	25·2	30·8	26·5	31·0
Mortality from birth to weaning, per cent.	46·1	31·1	27	36·3	29·6

Does were mated with male rats of the same group. When matings were non-productive the male was tested with a fertile female and the female with a fertile male. The percentages of fertile male and female rats were thus determined.

From these results Slonaker concluded that a diet in which 18 per cent. or more of the calories came from protein adversely affected reproduction by increasing sterility, delaying the birth of the first litter, shortening the breeding period and reducing the number of young and the number of litters born, but that a diet with 18 per cent. of calories from protein was best for prenatal development of the young and survival of the young to weaning. In general the higher levels of protein gave the best results during suckling as judged by the loss of weight of the mothers, the average size of litters nursed and the average weaning weights of the young. The largest average litter size at birth was obtained with the diet highest in protein.

In an earlier report Slonaker (1931*d*) was uncertain whether to attribute the increased sterility and lowered reproductive performance in rats on the diets with more than 14 per cent. of the calories from protein to some substance in the meat scrap which was used to increase the protein content of the diet, or to the increased protein content of the diet *per se*. He recommended, however, that for the good of both young and mother at least 26 per cent. of the energy of the maternal diet during lactation should be derived from protein.

In interpreting Slonaker's results it should be borne in mind that the reproductive performance obtained in rats on his diet with 14 per cent. of calories from protein compares unfavourably with that obtained on other diets by other workers. For instance, the experimental rats of Van Duyne *et al.* (1941), on a diet of the same protein content but of different composition (16 per cent. of dried whole milk) (see Table XII), on the average over

3 generations produced 44·9 young per female and *reared* 27·0 young per female, which is more than Slonaker's rats *bore* per female. Such a comparison may not be valid as has been pointed out earlier. The difference may not be dietary in origin, but the fact that Slonaker's does lost weight during lactation supports the view that his diet was not entirely satisfactory. If Slonaker's 14 per cent. protein diet was not adequate with regard to other constituents, the reduction in the percentage of these constituents resulting from the method adopted of increasing the protein content would, as Macomber (1934) has pointed out, accentuate deficiencies of other factors essential for reproduction if these were not present also in the meat scrap. Smith and Seegers (1934), unlike Slonaker (1931*a*), found no significant differences in the average number of young born per litter, average birthweight or weaning percentage in 3 consecutive litters of female albino rats maintained on diets containing 15 or 20 per cent. of protein. The diet used by these workers was inadequate in other respects for normal rearing of young. Macomber (1934) compared the effects on reproduction and lactation in rats of diets in which the protein content was varied from 5 to 21 per cent. by varying the proportion of casein to starch. His results will not be discussed here, however, because only two groups of does were used, one on the 21 per cent. protein diet and the other on diets containing 16·8, 10, 5 and 21 per cent. protein in successive reproductive cycles. His groups were therefore not comparable with regard to parity. Thompson (1935) found that the mean weight per pup at birth, and weaning, the percentage of stillbirths, the number weaned per litter and the weaning percentage in litters from rats given a diet containing 17 per cent. of protein from the beginning of pregnancy, were significantly better than when the diet contained 10 per cent. of protein. This is in agreement with Slonaker's findings for these protein levels.

Kellermann (1934) reported excellent results over a period of a year with a stock ration containing 0·6 per cent. of Ca and with 16·8 per cent. of the calories from mixed proteins; 90 per cent. of the litters of albino rats had an average weight of 40 to 60 g. per pup at an age of 23 days.

In a study of the nutritional requirements of pregnant and lactating rats by the self-selection method, Richter and Barelare (1938) found that rats with access to casein, sucrose, dried yeast, olive oil and salts chose a diet in which the percentage of calories from protein was 22·3 during the last 5 days before mating, 28·1 during the last 5 days of pregnancy and 23·8 during the last 5 days of lactation. In such studies the choice made may be influenced by the relative palatability of the purified foods offered in a somewhat uninteresting diet.

McCoy (1940) studied the effects of different levels of protein in the diet of the rat when the concentration of vitamins, fats and inorganic salts remained constant (Table X). A part of the dextrin of the basal diet was replaced by highly purified vitamin-free, salt-free casein to produce diets containing 15, 25 and 40 per cent. protein.

TABLE X

Reproduction and Rearing of Young by Rats on Different Levels of Protein Intake

Protein in Diet per cent.	No. Litters	Young	
		Total No. Born	Per cent. Successfully Weaned
15	227	1626	60
25	239	1816	77
40	283	2274	77

The average weaning weight was highest in the 25 per cent. and lowest in the 15 per cent. protein group.

Kao *et al.* (1941) made a study of the reproduction and lactation performance of 3 generations of albino rats on diets containing varying amounts of protein and Ca. They found that when the diet contained 0·6 to 0·8 per cent. of Ca in the air dried material there was no significant difference in the age at which female rats reached maturity, the average number of young born and reared per female and the duration of reproductive life on diets containing 18·8 and 25 per cent. of protein. The average weights of the young at 28 days were 40·2, 47·5 and 48·8 g. on diets containing 14·4, 18·8 and 25 per cent. of protein, respectively, and 0·6 per cent. Ca, the difference being more clearly significant between the 14·4 and 18·8 per cent. levels than between the 18·8 and 25 per cent. levels. They concluded that the optimum proportion of protein seemed to have been reached at a level of 18 per cent. in diets with the favourable Ca content of 0·6 per cent. in the dry food. The basal diet in these experiments was the Sherman diet A (or 16% of whole wheat 3 parts, dried whole milk 1 part, and NaCl in an amount equivalent to 2 per cent. of the wheat. This diet contains 14·4 per cent. protein and 0·19 per cent. Ca in the dry food. The increased protein content was obtained by including casein at different levels so that the proportion of whole wheat in the diet ranged from 69 to 78 and the dried milk from 13·8 to 15·6 per cent. The Ca and P contents were varied by adding Ca carbonate and phosphate. In these experiments, as in the experiments of Van Duyne, the reproductive capacity and ability to rear young were markedly superior to those of Slonaker's rats on the same dietary protein levels.

Schweigert (1947) reported preliminary results of replacing part of the wheat in the Sherman A and B diets by meat in reproduction tests with female rats mated with stock male rats.

In litter size at birth and average weight of young at weaning the group given diet A modified to contain one-sixth by weight of meat and containing 19·1 per cent. of protein was markedly superior to the group given diet A containing 15·2 per cent. of protein, and the group given diet B modified to contain one-sixth by weight of meat and containing 21·4 per cent. of protein was somewhat superior to the group given diet B with a protein content of 17·4 per cent. Work is in progress to determine whether the effect of including meat in the ration is due to increased protein content or to something else supplied by meat.

A recent preliminary communication from Sherman and Pearson (1947) indicated that increasing the protein content of the diet of female rats by addition of poultry meat to the Sherman diet A (or 16) reduced the age at birth of first young and increased the total number of litters and of young born, the number of young reared to weaning, the total and average weight of young at weaning, during an observation period covering the females up to 210 days of age. A nervous condition suggestive of Ca deficiency was observed in some females on the supplemented diet. Carcase analyses revealed low levels of body Ca in does which had reproduced on this diet. Experiments in progress indicate that does given a supplement of Ca as well as protein were healthier during the reproduction period than those receiving a protein supplement only.

It would appear from the above data that a diet containing 17 to 19 per cent. of suitable protein will meet the requirements of the albino rat for protein during pregnancy and lactation.

Nitrogen Balances

Rats

According to Morse and Schmidt (1944) Campbell in 1938 published a thesis on N balance in the rat during pregnancy and lactation. This thesis is not available for consultation but Campbell apparently found that rats maintained on a diet containing 2·4 per cent. of N

(15 per cent. of protein) stored amounts of N sufficient for the needs of the foetus, but that those on a diet containing only 1·2 per cent. of N (7·5 per cent. of protein) did not. During lactation the rats on the 15 per cent. diet lost N but were able to rear their young. At the lower level of protein rats were unable to rear their young unless there was an unusually large reserve of N at the beginning of lactation. Morse and Schmidt (1944) found that rats on a synthetic diet with 3 per cent. of liver extract and containing 3·8 per cent. of N (24 per cent. of protein) and rats on a stock diet containing 3·3 per cent. of N (21 per cent. of protein) had gained N at the end of pregnancy and in most cases at the end of lactation.

Body Protein

Rats

Poo *et al.* (1939; 1940) compared the protein content of the body before pregnancy, of rats reared on a diet containing 18 per cent. of protein with the protein content of the body during pregnancy just prior to delivery after 18 days on different levels of dietary protein (Table XI). The increased protein levels in the diet were obtained by increasing the casein at the expense of maize starch, the remaining constituents, lard, sardine oil, dry yeast, dry alfalfa and salts remaining constant.

TABLE XI

Effect of Increasing Protein Consumption During Pregnancy on Body Protein of Rats

	Before Pregnancy	During Pregnancy after 18 Days on Diets varied with respect to Protein			
Protein content of diet, per cent.	18	11	16	27	43
Protein eaten per rat per day, g.		1·04	1·95	2·89	4·44
Bodyweight, g.	150·000	191·333	232·301	228·394	207·100
Body protein content,* g.	23·546	26·245	31·518	31·594	29·885
Increase in body protein content,* g.		2·699	7·972	8·048	6·339
Ratio of protein eaten during 18 day period to increase in body protein		6·9	4·4	6·5	12·6
Ratio of calculated total food intake during 18 day period to increase in bodyweight		4·1	2·7	2·5	3·2

* The values for pregnant rats include protein content of young.

The data of Poo *et al.* and the ratios of protein intake to increment of body protein calculated from their data indicate most efficient use of protein at the 16 per cent. level. At the highest level of protein in the diet some of the protein is undoubtedly transformed into carbohydrate and used as a source of energy. Benditt *et al.* (1948) have just published a study on the effect on protein storage in young male rats of varying the protein and energy intake separately or together. A similar experiment on pregnant rats would clarify both energy requirements and optimum protein intake.

It would appear to be fairly well established that a level of 20 per cent. of suitable proteins in the diet is sufficient to support good reproduction and lactation in the albino rat.

This does not apply to purified diets containing as sole protein casein or some other protein relatively poor in cystine or methionine. The position with purified diets is further complicated by the interrelationship, not yet fully understood, of cystine, methionine, and choline (see also pp. 71, 72).

Sulpho-Amino-Acids and Levels of Casein in Lactation

Rats

Wilkinson and Nelson (1931) found that rats raised on a diet of casein 18, salts 3·7, butterfat 5·0 per cent., and soya bean at levels of 10 to 73 per cent. as source of the B vitamins, the diet being made up to 100 with dextrin, were unsuccessful in rearing their young. The mortality in the nursing young decreased with increased levels of soya bean in the diet. Supplements of liver and kidney caused marked improvement in lactation. Mapson (1932) reported poor lactation in rats on a diet containing as source of protein 23 per cent. of casein. The casein produced by another company gave inferior results to the former (Mapson, 1933) and was subsequently shown to be very low in cystine (Jones and Gersdorff, 1934). Soya bean protein is also known to be lacking in sulphur-containing amino-acids.

Kozlowska *et al.* (1932), using the prolongation of the lactation period as a means of studying the effect of nutritional factors on milk secretion in rats, found that a diet containing 10 per cent. of casein as source of protein was inferior to one containing 40 per cent. of casein, even when the food intakes of the rats were equalised.

Daggs (1934), Daggs and Tamboulian (1935) and Daggs (1935), using the increase in weight of rat litters between the 4th and 17th day as an index of the efficiency of lactation, found that in rats transferred just before parturition from a stock ration to a purified synthetic diet containing 12 or 15 per cent. of casein as sole source of protein, lactation was promoted by increasing the percentage of casein to 25 or by adding a supplement of cystine. Cysteine and methionine were also effective (Daggs and Lidfeldt, 1938). Sure (1941*a*) reported that female rats reared and bred on a purified synthetic diet of commercial casein 20, salts 4, dried yeast 10, fats 15, and starch 51 per cent., supplemented with cod liver oil and wheat germ oil, failed to rear their young (which were reduced at birth to 6 per litter) or reared only a small proportion of them. Inclusion of 0·2 per cent. of cystine in the mother's diet when the growth curves of the nursing young reached a plateau or " when abnormal gains were being made early in lactation," resulted in weaning percentages of 91 to 100.

Vinson and Cerecedo (1944) reported that female rats reared on a purified diet containing 20 per cent. of casein as source of protein failed to rear their young, but when the diet contained 30 per cent. of casein a moderately high proportion of young were reared. During lactation the does showed a sharp loss in weight which could not be prevented by increasing the percentage of casein in the diet above 30 but was prevented by addition of yeast. (See effect of folic acid, p. 77.)

Mueller and Cox (1946) using their own method of milking rats (Cox and Mueller, 1937) studied the effect of different levels of protein in the diet during lactation on the yield and composition of milk, on the number surviving to weaning and on the weaning weights of the young. The diets which were given to stock female rats from parturition consisted of dextrinised starch, lard 9·0 per cent. yeast concentrate 4·0 per cent., wheat germ oil 1·0 per cent., salts, carotene, rice cellulose, with casein at levels of 5, 10, 20, 30 and 50 per cent. The diet has been reported elsewhere to contain 0·245 per cent. of Ca and of P. The rats were milked on the 18th and 20th days of lactation. The percentage of fat in the milk decreased significantly as the casein content of the diet increased up to the 20 to 30 per cent. levels and thereafter increased. The trend in ash content was similar but the differences were not significant. No significant difference in protein content of milk resulted from differences in the protein content of the diet. The volume of milk produced by does on diets containing 20 and 30 per cent. casein was almost twice that of does on the 10 per cent. casein diet which in turn was about 6 times that of does on the diet containing 5 per cent. casein. The volume of milk at the 50 per cent. level of casein was slightly less than at 20 or 30 per cent. levels. The young reared by does on diets containing 20 per cent. or more of casein were about

one-third heavier at 21 days than the young of does on the diet containing 10 per cent. casein, which were in turn about one-third heavier than the young from the 5 per cent. casein group. These are approximations obtained from graphs, since the data are not tabulated. The numbers of young reared by does on the high and low levels of casein were slightly smaller than the numbers reared by does on the 20 and 30 per cent. levels.

The diets used by Daggs and his associates and by Sure contained yeast as source of the B complex and presumably were not deficient in choline. The diet used by Vinson and Cerecedo was a purified diet supplemented by synthetic vitamins and choline. It would appear from these investigations that a level of 25 to 30 per cent. of casein in a diet containing choline provides enough cystine for the normal lactation requirements of the rat (see also pp. 71, 72). Mueller and Cox's findings do not indicate any significant superiority of a 30 per cent. over a 20 per cent. level of casein under the conditions of their experiment.

Sure (1946) demonstrated the beneficial effect on the percentage of young reared by does of addition of 0·2 per cent. of cystine to their diet containing 8 or 9 per cent. true protein supplied solely by brewer's yeast or a cultured food yeast. Improvement was observed also when the percentage of yeast in the diet was increased to 40 per cent. so as to supply 16 to 18 per cent. true protein. These percentages of true protein were calculated from the N content of the yeast on the basis that 90 per cent. of the N was protein N.

Klose and Fevold (1947) found that when rats were reared from 40 days of age on a diet of maize starch, sucrose, cottonseed oil, salt mixture, cod liver oil and a mixture of synthetic vitamins including choline, with 37·5 per cent. of torula yeast as source of protein (equivalent to 20 per cent. crude protein in the ration) successful matings and pregnancies were obtained but only 12 per cent. of the young were reared to 21 days of age (average weight 18 g.). The inclusion of 0·2 per cent. cystine and methionine, respectively, in the ration in 2 comparable groups raised the percentage to 61 and 75. The average weights were 18 and 24 g., respectively. In a third group given 20 per cent. commercial casein as source of protein (equivalent to 17 per cent. crude protein) and yeast extract 5 per cent., the percentage young reared to 21 days of age was 86 (average weight 30 g.) compared with 96 per cent. weighing 27 g., in a stock diet control group. Third and fourth generation young were bred from first litters in the yeast and cystine group. The percentages of young reared to 21 days of age in the third and fourth generations were 77 and 96, respectively. The average weaning weight at 21 days in the latter was 26 g.

When pregnant 6-month-old females given a stock diet of purina dog chow, cod liver oil and liver prior to parturition were given the torula yeast diet during lactation the young survived but were only two-thirds the liveweight at weaning of young from control rats on stock ration throughout. The does on the yeast diet lost weight during lactation while the stock ration controls did not. The loss in weight of the does and retardation of growth of the young to weaning did not occur when the yeast diet was supplemented by cystine and methionine. Similar beneficial effects were obtained from supplements of cystine and of methionine in the lactation study when the source of protein in the basal ration was brewer's yeast. When casein 20 and brewer's yeast 2 per cent. supplied the protein in the ration during lactation there was a slight loss of weight in the does but the average weight of the young at 21 days was good. In all of these lactation studies in which the stock diet had been fed to parturition all the young survived to weaning.

Cystine has also been found to promote lactation when added at a level of 0·4 per cent. to a diet containing 40 to 50 per cent. of alfalfa leaf meal as sole source of protein (Wright and Haag, 1939; Smuts and Du Toit, 1941); the same was true of methionine and, to a lesser extent, cystine when added to groundnut protein (Haag and Wright, 1940).

Excess of Protein

SEXUAL MATURITY AND OVARIAN FUNCTION

Rats

Aschkenasy-Lelu and Tuchmann-Duplessis (1947) recently reported on the effect of a high-protein diet on somatic and sexual development in the young female rat. Female rats reared from weaning on a diet containing 90 per cent. casein grew more slowly and the opening of the vagina was delayed by 10 days compared with littermate sisters given only 18 per cent. of casein. The weight of the ovaries, total and per 100 g. bodyweight, was less after 26 days, approximately the same after 41 days and substantially greater after 64 days in the high protein group than in the lower protein group. Histological examination of the ovaries showed that after 26 days on the high protein diet the ovaries were immature, after 41 days the ovaries developed abnormally and after 64 days most of the follicles had disappeared and intense luteinisation was evident. (See also Fuchmann Duplessis and Aschkenasy-Lelu, 1947.)

REPRODUCTION AND LACTATION

Rats

Simmonds (1924) reported good reproduction and excellent lactation through several generations of rats on diets containing 31 to 67 per cent. of protein supplied as beef muscle, liver or kidney, and said to be adequate in other respects. Examination of the kidneys of rats on diets containing 31 to 40 per cent. of protein revealed severe kidney lesions, although there was no external evidence of loss of condition until they had been on the diet for 18 to 20 months.

McCoy (1940) obtained a higher average weaning weight of young from does receiving a diet containing 25 per cent. of protein compared with those receiving additional casein to give a protein content of 40 per cent. The weights are not given. Cowie and Folley (1947) report that uniparous female rats given a stock diet containing 20 per cent. protein from 5 months of age reared their litters from an average weight of 10·5 g. at 4 days to 42·0 g. at 21 days while a comparable group receiving the same diet with casein replacing wheat to increase the protein content to about 50 per cent. reared their young from an average weight of 9·0 g. at 4 days to only 31·0 g. at 21 days. In neither of these investigations did the high level of protein adversely affect the percentage of young reared. On the other hand in the experiment of Mueller and Cox (1940), as far as can be judged from the graphic presentation of their data, does on diets containing 50 per cent. of protein produced somewhat less milk and reared somewhat fewer young than does on 20 or 30 per cent. protein but the average weight of the young at 21 days was much the same in all 3 groups. Nelson and Evans (1947*a*) found no significant difference in reproduction when male and female rats were reared on a synthetic diet containing 24 per cent. casein as sole protein or on the same diet with casein replacing sucrose to give 64 per cent. of casein in the diet. But lactation on the high protein diet, as judged by the weaning weights of the young, was slightly better. This diet contained choline but was not supplemented by cystine.

It has been pointed out that the efficiency of utilisation of protein at a level of 43 per cent. in the diet of rats during pregnancy, as indicated by the ratio of protein intake to the increment of body protein, is much less than when the diet contains 16 per cent. protein, probably because some of the protein is transformed into carbohydrate and used as a source of energy (see Table XI).

To sum up in general terms it may be said that a level of 20 per cent. of mixed proteins in the diet of the rat is adequate for good reproduction and lactation. In purified diets containing casein as sole protein and containing choline the level of protein must be stepped

up to 25 to 30 per cent. unless a supplement of cystine or methionine is included. The usual amount of supplement appears to be 0·2 per cent. of cystine. When the diet contains 40 to 50 per cent. of protein, efficiency of protein utilisation is probably impaired. Very high levels of protein in the diet appear to have an adverse effect on the sexual development of the female rat.

The requirements of other species of laboratory animals have not been studied.

Parental Protein Intake and Sex Ratio in the Young

Rats

There is some evidence that the sex ratio of the offspring may be influenced by the protein content of the parents' diet. Hoelzel *et al.* (1939) obtained a sex ratio of 145 males : 100 females in the young produced as a result of the mating of male rats on high levels of protein (30 or 50 per cent.) with female rats on low levels (7·5 or 12 per cent.) and a ratio of 92 : 100 when the diets of the male and female parents were reversed.

But other factors are involved. Slonaker (1938), and Murray (1941) reported sex ratios differing widely from that reported by Asdell *et al.* (1941) for the young of rats reared on diets containing approximately the same level of protein.

CARBOHYDRATE

Rats

There is no direct evidence that carbohydrate as such is necessary in the diet of the rat. Follis and Straight (1943) studied the effect of a carbohydrate-free diet on a group of 12 albino rats. Only 2 of the rats were female. One produced a litter and destroyed it, the other produced and reared an apparently normal litter. Of the 2 female rats in a control group receiving the same diet but with 10 per cent. of sucrose substituted for 5 per cent. each of protein and fat, one produced a litter and destroyed it, the other was found to have foetuses *in utero* at autopsy.

Whitnah and Bogart (1936) reported that replacement of 20 per cent. of starch by sucrose in a ration consisting of yeast, salts, cod liver oil, casein, butter oil and starch resulted in earlier maturing of female rats. These, when given a stock ration and mated with normal male rats failed to reproduce.

CALCIUM, PHOSPHORUS AND VITAMIN D

Restricted Supply of Calcium

reproduction and lactation

Rats

McCollum *et al.* (1922) found that rats reared on a basal diet of cereals, peas and beans, casein, butterfat, NaCl and $NaHCO_3$, containing 0·050 per cent. of Ca and 0·400 per cent. of P, were sterile. A similar diet with added $CaCO_3$, containing 21 per cent. of protein, 0·10 per cent. of Ca and 0·4 per cent. of P resulted in a very low level of reproductive and rearing ability in female rats. When the Ca content of the diet was stepped up to 0·26 per cent. by further additions of $CaCO_3$, the performance progressively improved. The critical level of Ca necessary to allow growth and reproduction over several generations appeared to be about 0·22 per cent., but it is stated that 0·65 per cent. Ca was necessary for normal growth, reproduction, rearing of young and longevity. These results were confirmed by Sherman and his co-workers (Campbell *et al.*, 1935 ; Sherman and Campbell, 1937 ; Van Duyne *et al*, 1941 ; Kao *et al.*, 1941). They found that a diet containing 16·7 per cent. of protein, 0·192 per cent.

TABLE XII

AVERAGE REPRODUCTIVE PERFORMANCE OF RATS ON MODIFIED SHERMAN A DIET OVER 3 GENERATIONS

	Sherman and Campbell 1937		Van Duyne *et al.* 1941		Kao *et al.* 1941				
Diet	A	162	168	169	170	171	172	673	675
Construction of Diet	1·6 Whole Milk Powder, 5·6 Ground Whole Wheat NaCl 2 per cent of Weight of Wheat	A + $CaCO_3$	A + $CaCO_3$	A + $CaCO_3$ & $CaHPO_4$ $2H_2O$	A + Casein	A + Casein + $CaCO_3$	A + Casein + $CaCO_3$ + $CaHPO_4$	A + Casein + $CaCO_3$	A + Casein + $CaCO_3$ + $CaHPO_4$
Protein (percentage in air-dried food)	13·1	13·0	13·4	13·3	18·8	18·8	18·8	25·0	25·0
Ca " " "	0·195	0·337	0·64	0·8	0·19	0·61	0·77	0·61	0·77
P " " "	0·4	0·4	0·4	0·53	0·4	0·4	0·53	0·46	0·56
Ca : P	0·5	0·8	1·5	1·5	0·5	1·5	1·5	1·5	1·5
Cal. per g. air-dried food	3·73	3·71	3·69	3·67	—	—	—	—	
No. of female rats	101	102	105	104	—	—	—	—	—
No. bearing young	90	98	—	—	11	40	33	21	6
Percentage bearing young	89	96	—	—	—	—	—	—	—
Age in days at birth of first young	132	111	111	110	124	102	104	101	100
Reproductive span in days	213	279	311	360	267	319	378	328	302
No. of young born per female	24·1*	32·6*	38·6*	44·9*	36	43	48	44	45
No. of young reared per female	12·7*	20·4*	24·7*	27·0*	23	29	32	28	34
Percentage of young reared	46·0	56·6	—	—	64	69	66	63	76
Average weight of young at 28 days, g.	38·9	42·4	40·2	40·1	44·5	47·5	46·9	48·8	48·4

*These figures are based on total number of females in group, not on number of females bearing young.

of P and 0·094 per cent. of Ca in the air-dry state, when fed to female rats from weaning, resulted in poor reproduction and rearing ability in the first generation, and complete inability to rear young and premature sterility in the second generation. A level of 0·195 per cent. of Ca in a diet containing 13 per cent. of protein and 0·4 per cent. of P on an air-dry basis enabled reproduction and rearing of young to take place over 21 generations, but performance was progressively improved when the Ca content was stepped up to 0·337, 0·64 and 0·8 per cent. in comparable groups. The P content remained at 0·4 per cent. except in the diet highest in Ca, where it was increased by addition of $CaHPO_4$ to give the same Ca : P ratio (1·5) as in the diet containing 0·64 per cent. Ca. At levels of 18·8 and 25·0 per cent. of protein and with a Ca : P ratio of 1·5 there was little difference between the reproductive and rearing capacity of groups receiving 0·6 and 0·8 per cent. of Ca in the diet (Table XII).

Campbell and Sherman (1945) recently repeated their investigation of the effect of varying levels of Ca in the diet, using a basal diet which contained a higher proportion of milk and was therefore richer in protein, riboflavin and vitamin A, and which had been shown to give better results in reproduction studies (Sherman and Campbell, 1924) than the basal diet A. This diet (diet B) consisted of one-third dried whole milk and two-thirds ground whole wheat with NaCl added at a level of 2 per cent. of the weight of wheat, and contained 16 per cent. of protein, 0·34 per cent. of Ca and 0·5 per cent. of P in the air-dry food, with an energy value of 4 Cal. per g. The Ca content was raised to 0·48 and 0·64 per cent. by addition of $CaCO_3$. In 2 generations of albino rats maintained on these diets, the average age at which female rats bore their first young and the average size of litters at weaning were not significantly different. The length of the reproductive life and the number of young reared per doe (Campbell and Sherman, 1945) and the average weaning weight (Lanford *et al.*, 1941) are given in Table XIII.

TABLE XIII

Effect of Level of Ca in the Diet on Average Reproductive Performance of Female Rats

Percentage Ca in diet	0·34	0·48	0·64
Duration of reproductive life (days)	331±10·6	355±11·5	333±11·6
No. of young reared per female	29·6±1·21	32·3±1·37	30·0±1·31
Average weight of young at 28 days—Males g.	43·3	43·3	41·8
Females g.	42·6	41·6	40·0

Since none of the differences was more than 3 times the probable error and the number of experimental animals was fairly large, Sherman and Campbell concluded that, with a diet sufficiently good in other respects, a range of 0·34 to 0·64 per cent. of Ca represents a plateau of optimal intake. Analyses of representative young, however, showed that the percentage of Ca in the body at weaning increased significantly with each increase in the level of Ca in the diet (Lanford *et al.*, 1941).

Restricted Supply of Phosphorus

Sexual maturity and oestrous cycle

Rats

Guilbert and Hart (1930) reared female rats from weaning on a diet containing 1·5 per cent. of $CaCO_3$ and 1·3 per cent. of KH_2PO_4 and on the same diet with the phosphate replaced by an equal amount of dextrin. Removal of the phosphate reduced the P content of the

diet from 0·557 per cent. to 0·220 per cent. The Ca content of both diets was 0·825 per cent. Reduction of the P content had an adverse effect on weight increase, delayed the onset of sexual maturity and caused complete cessation of oestrus. Irregularity or cessation of oestrus was observed also when this low-P diet with a Ca : P ratio of 4 : 1 was substituted for a stock diet at sexual maturity (on the average about 50 days of age). Regular cycles were resumed when the Ca content was reduced to the level of the P or when the P level was increased to 0·557 per cent. Regular cycles, however, were also resumed a few days later by the rats on the low P diet (Ca : P ratio 4 : 1) when they reached 100 days of age, although no change in the diet had been made. Subsequent addition of $CaCO_3$ to give a Ca : P ratio of 8 : 1 resulted in suppression of oestrus. The oestrous cycles of rats raised to about 110 days of age on a stock diet and then given the diet containing 0·22 per cent. of P and 0·825 per cent. of Ca were not disturbed.

Pallot and Mouriquand (1933) observed that young female rats given a high-Ca rachitogenic diet at a liveweight of 40 to 50 g. ceased to ovulate from the twelfth day.

OVARIAN AND TESTICULAR DEVELOPMENT

In a recent histological study of the tissues of rats on a diet low in P, Follis *et al.* (1941) found that in male and female rats given a diet containing only 0·017 per cent. of P with 0·4 per cent. of Ca and a supply of vitamin D the testes and ovaries did not develop normally as compared with a control group receiving a supplement of P to raise the P content of the diet to 0·27 per cent. Spermatogenesis ceased within about 30 days and the onset of oestrus was retarded. Only half of the females ovulated. However, the results of a paired feeding experiment in which the food intake of the control group on the supplemented diet was limited to the amount eaten by the low-P group indicated that these effects could be attributed to inanition rather than to a specific effect of P deficiency. Rats

Comparison of Different Levels of Calcium and Phosphorus in the Diet

Records of Performance

The effect of varying the amounts of Ca and P in the diet absolutely and relatively was studied by Cox and Imboden (1936*a*) in relation to the reproductive and lactation performance of the rat over 10 consecutive reproductive cycles. All the female rats used had successfully produced one litter on a stock ration. The basal diet consisted of casein (acid-washed) 20, dextrinised starch 56·1 to 55·1, lard 6·0, yeast concentrate 3·0, wheat germ oil 1·0, carotene solution in oil 0·3, salts 3·1 and rice cellulose 1·2 to 5·0 per cent., and contained 0·017 per cent. of Ca and 0·245 per cent. of P. Vitamin D was not supplied either directly or indirectly. The Ca and P levels were varied by addition of suitable proportions of dicalcium phosphate and monobasic ammonium phosphate or calcium acetate. A Ca : P ratio of 1·0 at a Ca level of 0·100 per cent. was considered, on the basis of weight of young at 21 days, ash content of the young and change in weight of the mothers, to be the ideal ratio and level for successful gestation and lactation in the rat. The poorest results were obtained with diets containing 2·450 per cent. of Ca with Ca : P ratios of 10·0 and 5·0. Poor results were obtained also with diets containing 0·017, 0·245, 1·225 and 2·450 per cent. of Ca when the Ca : P ratios were respectively 0·07, 0·1, 5·0 and 3·33. The diet which most nearly approached the optimum level of 0·40 of Ca and 0·40 per cent. of P contained 0·735 per cent. of Ca and 0·50 of P with a Ca : P ratio of 1·5. Data are presented also for the percentage of fertile matings and the percentage weaned (litters were reduced to 6 at birth), although these have not been taken into account in the above assessment. The percentage of fertile matings was higher on the Rats

diet containing 0·735 per cent. of Ca with a Ca : P ratio of 1·5 than on the diet with 0·49 per cent. of Ca and Ca : P ratio 1·0, but the reverse was true of the weaning percentages. At Ca levels of 0·245, 0·49 and 0·735 per cent., there was, in general, a progressive decrease in percentage of fertile matings and weaning percentage with increasing levels of P in the diet. The greatest average number of litters was borne by the rats on the diet containing 0·735 per cent. of Ca and 0·49 per cent. of P.

Bodansky and Duff (1941*a*) who used several of Cox and Imboden's diets in a study of parathyroid function in pregnant rats, compared the percentages of fertile matings obtained in normal rats on these diets with the values given by Cox and Imboden. The diets fell roughly into the same order according to the percentage of fertile matings, the principle exception being the diet containing 0·49 per cent. of Ca and P, which came lower in their list than it did in that of Cox and Imboden. The diets containing 0·49 and 1·225 per cent. of Ca with Ca : P ratios of 0·66 and 1·0, respectively, gave the highest percentage of fertile matings (100). The former diet and the diet containing 0·122 per cent. of Ca with a Ca : P ratio of 0·5 gave the largest average number of young per litter and the largest average litter weights at birth and the average pup weights were among the lowest recorded. The average birth-weight per pup was slightly higher on the diet containing 0·735 per cent. of Ca and 0·49 per cent. of P (Ca : P ratio 1·5).

Bodansky and Duff (1941*b*) also noted that the diet containing 0·735 per cent. of Ca and 0·49 per cent. of P produced young which were somewhat heavier than the offspring in other diet groups and contained higher percentages of solids, ash, Ca and P. It is doubtful, however, if their groups were strictly comparable, one with another, with regard to litter parity. They were certainly not strictly comparable with the groups of Cox and Imboden.

Mueller and Cox (1946) investigated the effect of the Ca and P content of the diet of rats during lactation on the volume and composition of the milk. The diets used were a selection from the series used by Cox and Imboden (1946*a*). Rats were given the experimental diets from parturition and were milked on the 18th and 20th days of lactation. The results were presented graphically. When the Ca content of the diet was 0·245 per cent., increasing the P content from 0·245 to 2·450 per cent. did not much affect the volume or the percentage of ash, Ca and P in the milk. Nor was the weight of the mothers or of the young adversely affected. When the P content was 0·245 per cent., increasing the Ca content from 0·49 to 2·450 resulted in a progressive decrease in volume of milk and percentage of ash, Ca and P and in the total amount of Ca secreted ; an increase in the Ca : P ratio of the milk ; a progressive increase in the weight lost by the mother and a progressive decrease in the weight of the young at 21 days of age.

Of the diets studied by Mueller and Cox, that containing 0·49 per cent. of Ca and 0·245 per cent. of P, Ca : P ratio 2·0, gave the best results in terms of milk production. It is unfortunate that the diets which permitted the most successful reproduction performance in the investigation of Cox and Imboden (1936*a*) and Bodansky and Duff (1941*a*) were not included in the series used by Mueller and Cox.

Gaunt *et al.* (1939) showed that increasing the Ca and P content of a poor human diet fed to female rats, from 0·092 and 0·226 to 0·261 and 0·305 per cent., respectively, of the dry diet resulted in improved but not optimum reproduction and ability to rear young in 3 generations of first litters, the difference being especially marked in the second and third generations.

The optimum level of Ca in the diet for reproduction and lactation would appear to be anything from 0·3 to 0·8 per cent., depending on the quality of the diet in other respects. At such levels of Ca the optimum Ca : P ratio probably lies between 1·0 and 2·0.

Calcium and Phosphorus Balances

Rats

Goss and Schmidt (1930) studied the Ca and P balance of female rats on mixed diets containing 0·74 to 0·82 per cent. of Ca and 0·50 to 0·53 per cent. of P during 2 to 4 successive reproductive cycles separated by rest periods. Out of a total of 27 pregnancies, there was failure to store Ca in only 4, and in only 3 was there a negative P balance. In all of the 17 lactations observed, the rats were in negative Ca balance. Negative P balances occurred in 16 lactations and P equilibrium in one. All but 2 changed from negative to positive Ca and P balances following weaning of the young.

Foetal Supply and Maternal Reserves of Calcium and Phosphorus

Rats

Among the diets tested by Cox and Imboden (1936*a*), those containing 2·450 per cent. of Ca with Ca : P ratios of 3·33, 5·0 or 10·0 were the only ones which resulted in significantly lower birthweights and percentages of Ca and P in the second litter young. Other diets, notably the diet containing 1·225 per cent. of Ca with a Ca : P ratio of 5·0, resulted in a lowered ash content of young after several successive cycles (Cox and Imboden, 1936*b*). The ability of the maternal organism to regulate the supply of minerals to the foetus in the first and second pregnancy on a low Ca diet, as indicated by the results of Cox and Imboden (1936*b*), is consistent with the findings of earlier workers (Toverud, 1923; Korenchevsky and Carr, 1923; Macomber, 1927; Sherman and Booher, 1931) and was confirmed by Bodansky and Duff (1941*b*) who showed that the total Ca in the litters produced by rats on diets very low in Ca might amount to 3 or 4 times the total dietary intake of Ca of the mother during gestation. They also showed by means of balance studies that rats on very low Ca diets utilised their dietary Ca much more efficiently than at higher levels of intake. Bodansky and Duff suggest that in subsequent litters (if the diet supplies enough Ca to carry the rat through subsequent gestations), as the maternal reserve of Ca is reduced, further loss is minimised by a reduction in the number of young per litter. Boelter and Greenberg (1943) found a much lower percentage of Ca at birth in stillborn rats than in young born alive to mothers on a diet very low in Ca, indicating that only those foetuses which had an almost normal Ca content were carried successfully to term. They found that the percentage of ash in the bones of the young in the low-Ca group was much lower at birth and throughout suckling than in the group receiving a supplement of Ca. The bodies of the mothers were very low in Ca at the end of lactation compared with those in the supplemented group.

The foetal store of Ca in the young of rats given a diet containing 0·49 per cent. of Ca with a Ca : P ratio of 1·0 amounted to only 10 per cent. of the total Ca consumed by the mother during pregnancy. The young of rats on such diets show a much better ash content at weaning than do the young of rats on low-Ca diets whose body reserves of Ca are reduced by the demands of the foetus during gestation (Cox and Imboden, 1936*a*, *b*; Gaunt *et al.*, 1939).

In a recent study of the effect of pregnancy on the P turnover of the skeleton of rats maintained on normal and rachitogenic diets, Manly and Levy (1941) showed by using radioactive P that there was no marked loss of inorganic tooth or bone substance during pregnancy.

The strain of lactation on the mother's reserves of Ca has been demonstrated by Sherman and Macleod (1925), who found that, on a diet of whole milk powder, wheat and NaCl, containing 0·34 per cent. of Ca and 0·5 per cent. of P, female rats which had reared young had lower absolute and relative amounts of Ca in their bodies than those which had not reared young. On the average there was a reduction of the percentage of body Ca by the end of the first or second lactation, after which there appeared to be an approximate balance between

the tendency to lose Ca during lactation and the tendency to regain it during the intervening period. This balance would be influenced possibly by the interval allowed between weaning and subsequent mating.

Mice — Pecher and Pecher (1941) demonstrated by means of radio-active Ca, that Ca previously fixed in the skeleton of mice migrates to the foetus during the last days of pregnancy and to the offspring through the milk.

Rats — Warnock and Duckworth (1944) recently observed that in rats on an adequate diet, withdrawal of bone salt during lactation is restricted to the spongiosa of the long bones, the shaft being unaffected. They suggest that lactation requirements for Ca and P could be defined as those intakes which for a given output of minerals in milk will restrict the withdrawals to the spongiosa. On an adequate diet the rate of replenishment of the depleted spongiosa at the end of lactation was the same whether the animals were then pregnant or not.

Influence of Vitamin D

oestrous cycle

Rats — Richter and Rice (1943) found that when young female rats were transferred at 60 days of age from a stock ration to a diet of maize only, they showed within 12 to 50 days continuous di-oestrus or only very rare cornification of the vagina. Ultraviolet irradiation or daily administration of 50 U.S.P. units of vitamin D_3 restored normal cycles. They concluded that vitamin D probably brought about a more efficient utilisation of the small amounts of Ca present in maize. (The approximate Ca and P content of such a diet would be of the order of 0·01 per cent. of Ca and 0·27 per cent. of P.) Boelter and Greenberg (1943), however, reported that when immature female rats weighing less than 100 g. were transferred from stock diet to a purified diet containing 0·01 per cent. of Ca and 0·425 per cent. of P, they showed continuous di-oestrus and failed to mate although the ration included 2·27 per cent. of cod liver oil. When adult female rats were transferred to this deficient diet they showed normal oestrous cycles. In 26 female rats each of which had produced a litter on a stock ration before being transferred to the Ca-deficient diet, production of young occurred in only 14 after 48 attempts at mating, and only 6 produced live young. The average liveweight at birth was the same as for the young of control mothers which received a supplement to bring the Ca content of the diet up to 0·546 per cent., but the control young grew better during the suckling period.

Records of Performance

Rats — McCollum *et al.* (1922) and Simmonds (1924) found that when their low-Ca diets contained 1 to 3 per cent. of cod liver oil the fertility and percentage of young weaned by rats on these diets was greater than when the diets contained butterfat, usually 8 per cent., as source of fat, but the difference tended to disappear as the Ca content approached 0·26 per cent. Simmonds says that female rats on low-Ca diets containing cod liver oil are apparently successful in nourishing their young but are likely to collapse after they have nursed 2 litters. These workers considered 0·65 per cent. of Ca and 0·40 per cent. of P in the diet to be the optimum amounts for the rat during reproduction and lactation, because when the diet contained these amounts they were unable to demonstrate any superiority of cod liver oil over butterfat.

It is perhaps of interest to note the results obtained by Sherman and Campbell (1937) in an attempt to identify the constituents of milk responsible for the superior reproductive performance of rats on diet B (one-third whole milk powder to two-thirds whole wheat with

added NaCl) over that of rats on diet A (one-sixth whole milk powder to five-sixths whole wheat with added NaCl). They found that the reproductive performance of rats on diet A supplemented with butterfat to give a fat content equal to that of diet B was similar to that of rats receiving diet A supplemented with $CaCO_3$ to give a Ca content equal to that of diet B. Addition of both butterfat and Ca did not result in any further improvement. They concluded that both Ca and vitamin A were concerned in the beneficial effect resulting from increasing the proportion of milk in the diet (see also page 55). The beneficial effect of the butterfat supplement was observed on the low-Ca diet only; it is possible that additional vitamin D provided by the butterfat may have resulted in increased utilisation of Ca.

Recently Embleton and Collings (1947) reported that irradiation of the 10 per cent. whole milk powder component of their stock diet prevented the usual seasonal drop in the numbers of litters born in their rat colony during the winter months of the year.

Calcium and Phosphorus Balances

Toverud and Toverud (1931; 1932) found that bitches on a diet low in Ca and P showed negative balances of Ca and P at the end of gestation, and much larger negative balances at the end of lactation, irrespective of whether vitamin D was supplied as cod liver oil in the diet or not. Dogs

Foetal Supply and Maternal Reserves of Calcium and Phosphorus

In the studies of Toverud and Toverud (1931; 1932) reported above, the ash, Ca and P contents of the maternal bones at the end of lactation and of the bones and bodies of the young at birth were below normal, irrespective of whether vitamin D was supplied as cod liver oil in the diet of the mother during pregnancy and lactation or not. Nor did the addition of vitamin D in cod liver oil to the mother's diet protect the pups against the occurrence of bone abnormalities when weaned to the maternal diet with or without cod liver oil. The diet used in these studies supplied 10 to 17 mg. Ca and 16 to 40 mg. P per kg. bodyweight, depending on the weight of the bitch used, and the Ca : P ratio ranged from 0·4 to 0·7. Dogs

Nicholas and Kuhn (1932) investigated the effect of giving viosterol daily from mating to female rats on a mixed diet containing 0·97 per cent. of Ca and 0·50 per cent. of P and on a diet of wheat flour and maizemeal containing only 0·13 per cent. of Ca and 0·20 per cent. of P. The addition of viosterol led to the production of larger young containing greater total amounts of Ca and P. The percentages of ash and Ca in the bodies of the young were also higher in the viosterol groups, and the mothers' bones had a higher percentage of Ca and P. The effect of the viosterol was seen most clearly in the group on the diet with the higher levels of Ca and P. Nicholas and Kuhn concluded that viosterol has a dual role; it causes better assimilation of Ca and P from the diet by the mother, and better transmission of Ca and P from the mother to the foetus. Rats

Swanson and Iob (1935) found that addition of viosterol to a diet containing 0·418 per cent. of Ca and 0·302 per cent. of P consumed by female rats gave a significant increase in the percentages of Ca and P in the young at birth as compared with the young of mothers whose diet was not supplemented with vitamin D. This result appeared to be true also if the Ca and P contents of the young were expressed as storage per pup or per litter. The results of Sontag *et al.* (1936) showed similar increases in the percentage and total Ca and P contents of pups of female rats given vitamin D as supplement to a diet low in Ca and P, but because of the larger litter size in the unsupplemented group the total Ca and P output of the mother was less in the supplemented group than in the unsupplemented group.

It is of interest to note that Sinclair (1942), in continuation of the work of Bodansky and Duff (1941*a*, *b*), found that Cox and Imboden's "optimum diet" containing 0·49 per cent. of Ca and P failed to prevent parathyroid enlargement in the rat during pregnancy, with cumulative effects in a second pregnancy. They suggest that if enlargement of the parathyroid gland is an indication of dietary deficiency this diet is inadequate during pregnancy. When 100 units of viosterol were added daily to this diet, enlargement of the parathyroids was not so marked. In the group of rats receiving Cox and Imboden's diet containing 0·122 per cent. of Ca and 0·245 per cent. of P, maternal parathyroids were larger and foetal parathyroids smaller than in the group on the "optimum diet." Addition of viosterol reduced the amount of enlargement of the maternal parathyroid gland which occurred during pregnancy, and increased the size of the foetal gland. The greatest enlargement of the parathyroid occurred on the very low Ca diet (0·017 per cent. of Ca and 0·245 per cent. of P).

According to Reed *et al.* (1939), Foa showed in 1936 that irradiation of pregnant rats produced an increase of 21·2 per cent. in the CaO and an increase of 19·2 per cent. in the P_2O_5 content of the embryos.

Rats

The beneficial effect of vitamin D in correcting an adverse Ca : P ratio in the diet of growing rats is well known. McCollum *et al.* (1927) showed that when female rats were given cod liver oil from before mating, their young were protected to some extent against rickets when subjected later to a rachitogenic diet. A similar protective effect when the stock diet of the does contained irradiated milk powder in place of ordinary milk powder was observed by Embleton and Collings (1947).

Ssamochwalowa and Iwanowa (1933) showed that ultraviolet irradiation of rats receiving a rachitogenic diet during pregnancy and lactation reduced neonatal mortality and prevented the occurrence of rickets in the young and, according to Warkany (1943), addition of vitamin D to a rachitogenic diet with a high Ca : P ratio prevented the occurrence of foetal rickets.

Rabbits

Nachtsheim (1930-31) observed that when rex furred rabbits were crossed with normal coated rabbits in such a way that the same litters contained both normal and rex furred young the latter were more susceptible to rickets. Wilson (1934) noted also the occurrence of bone abnormalities in the second generation of young from rex and normal crossing, the condition being more prevalent in the rex furred offspring and most common in large litters. Nachtsheim found that the tendency to rickets in the rex furred young could be counteracted by giving to the mother from the day of parturition, and subsequently to the young also, a mixture containing Ca, P and vitamin D.

To sum up, it would appear that at very low levels of Ca intake the inclusion of vitamin D in the diet does not counteract the depressing effect of the Ca deficiency on the ability of the female to mate and produce live young. At higher levels of intake which are still suboptimal for reproduction, addition of vitamin D to the diet appears to increase the female's ability to rear her young, and addition of vitamin D to a rachitogenic diet with a high Ca : P ratio prevents the occurrence of foetal rickets. In the absence of suitable experimental data it is impossible to say whether the optimum level of Ca and the optimum Ca : P ratio for rats in the absence of vitamin D would be the same if vitamin D were supplied in the form of concentrates in the diet or by ultraviolet irradiation.

Excess of Vitamin D

Rats

Nicholas and Kuhn (1932) and Swanson and Iob (1935) reported that large doses of vitamin D as viosterol had an adverse effect on the course of pregnancy in rats. Guldager (1936) is quoted by Reed *et al.* (1939) as having observed arteriosclerosis in maternal and foetal

placentas and sometimes death of the foetus when pregnant rats were overdosed with vitamin D. Ssolianikov (1938) observed persistent cornification of the vagina in mature female rats on a normal diet supplemented with large doses of vitamin D.

MAGNESIUM

Comparison of Different Levels of Magnesium in the Diet

Rats

Tufts and Greenberg (1938) found that rats reared on a diet containing 0·39 per cent. of Ca and 0·45 per cent. of P, or on a diet containing 0·87 per cent. of Ca and 0·80 per cent. of P with 5 mg. Mg per 100 g. of food were able to breed successfully. Many of the mothers refused to nurse their young, and litters which were reared to weaning did not show the increase in body Mg shown by young suckled by mothers on a diet higher in Mg. They grew well and, apart from the hyperirritability typical of Mg deficiency, appeared normal. Tufts and Greenberg concluded that the mother's milk was deficient in Mg. In the control group on the same diet with Mg sulphate added to give a Mg content of 50 mg. per 100 g. of diet, the bodies of the young at 21 days were somewhat lower in Mg than those of stock young of the same age, indicating that even this was not an optimum level of Mg. When the Ca and P contents of the diet were increased to 1·16 and 0·75 per cent., respectively, by suitable additions of Ca phosphates, reproductive performance was poor in both low-Mg and control groups. Few of the animals in the low-Mg, high-Ca group survived long enough to breed, and those that did survive were in too poor condition. Increasing the Mg content to 13 mg. per 100 g. diet in another group of female rats receiving the same basal diet with 1·66 per cent. of Ca and 1·0 per cent. of P resulted in positive evidence of pregnancy in almost all cases, but in each the litter was either resorbed or, if born, was eaten immediately after birth. This happened also in the control group on the high-Ca diet with a Mg content of 50 mg. per 100 g. food.

These results suggest that female rats bred on a diet with optimum levels of Ca and P and 50 mg. Mg per 100 g. of food can successfully bear and rear first litters; but since the young of these litters at weaning contain less Mg per cent. than stock young, further investigation is needed to determine the effect of this intake of Mg over several reproductive cycles and for several generations.

IODINE

Requirement

Rats

The minimum level of I in the diet compatible with successful breeding performance in rats is not known. Remington (1937) found that male and female rats on a goitrogenic diet could reach maturity and produce normal numbers of living young, despite almost complete absence of I and colloid from the thyroid gland, and according to Warkany (1945), Nelson and Warkany have interbred such goitrous rats for 5 generations without observing any sign of injury to the young. Kellerman (1934) has reported excellent breeding results with rats on a stock diet containing 33 μg. I per 100 g. ration.

Excess of Iodine

Rats, Guinea-pigs, Mice

Buchheim (1932) and Lipschütz and Morales (1936) confirmed the findings of earlier workers that excessive intake of I may cause underdevelopment of the seminal vesicles in rats, guineapigs and mice. Under the conditions of their experiments spermatogenesis was normal. Lipschütz and Morales found no evidence of testicular atrophy in young rats given

10 mg. I daily in milk for 2 or 3 months. Buchheim gave daily doses of 10, 35 and 2·5 mg. I in milk to rats, guineapigs and mice, respectively, for periods varying from 8 days to 4 months or longer, and observed that secondary sex characters did not develop in young animals and showed regression in adults. Sueda (1937) reported that large doses of KI adversely affected fertility in male rats.

IRON, COPPER AND MANGANESE

Rats

Complete failure of does to reproduce, and a high proportion of male sterility, were observed in rats reared on an exclusively whole milk diet when matings with stock animals or milk-fed animals were attempted (Mattill and Conklin, 1920; Mattill and Stone, 1923; Palmer and Kennedy, 1922-23, and Krauss, 1931). Early efforts to improve the value of a milk diet for reproduction and lactation were reviewed by Van Donk *et al.* (1933). These and subsequent investigations referred to below show a lack of agreement on the effect of a diet of milk with added Fe and Cu on the oestrous cycles of female rats and on the fertility of male rats, but all the investigations show that where female rats on such a diet were successfully mated with stock male rats, litters considered to be of approximately normal size and birthweight were obtained but the young were not reared. Addition of Mn to a diet of milk fortified with Fe and Cu resulted in the rearing of the young. It is clear, therefore, that experiments in which Fe and Cu but not Mn have been added to a basal milk diet can be discounted in any attempt to assess the Fe and Cu requirements of the female rat for reproduction and lactation.

The effects of varying the amounts of Fe and Cu added to a milk diet containing a constant amount of added Mn have apparently not been investigated and in experiments designed to test the effect on rats of adding different amounts of Mn to a diet of milk supplemented with Fe and Cu, quite arbitrary levels of fortification with Fe and Cu appear to have been used by different workers (see Table XIV).

The interpretation of results obtained when supplements of Fe, Cu and Mn are added to a basal diet of liquid milk is complicated by the fact that on such diets the energy intake is inadequate (Cunningham, 1931; Skinner *et al.*, 1932; Van Donk *et al.*, 1933).

Comparison of Different Levels of Iron in the Diet

Breeding Performance

Rats

Keith and Miller (1933) found that the production and rearing of first litters by the first and second generation of female rats on a solid diet of dried skimmed milk, lactose, starch, fats, NaCl, cod liver oil, yeast and wheat germ oil, with an Fe content of 0·0001 per cent., was much improved by doubling the Fe content, the difference being especially marked in the second generation. It is unfortunate that the increased Fe content was not obtained by simple addition of an Fe salt but by the inclusion of 20 per cent. dried beef muscle in place of an equicaloric amount of fat; the authors aver that the improved performance could not be attributed to the increase in protein content of the diet from 15 to 27 per cent., since they considered the former to be the optimum level of protein.

Foetal Supply of Iron

Rats

Alt (1938) showed that female rats restricted to a diet of dried whole milk from the time of first mating produced first litters of normal size and weight but containing only half the amount of body Fe per newborn pup found in first litters of does on stock diet or in litters

TABLE XIV

EFFECTS OF MANGANESE SUPPLEMENTS ON REPRODUCTION AND REARING OF YOUNG RATS

Basal Diet	Mn Supplement	Mn Intake per Rat Daily	Female Rats: Oestrous Cycle	Female Rats: Rearing of Experimental Young by Experimental Females (Successful +)	Female Rats: Rearing of Stock Young by Experimental Females (Unsuccessful −)	Female Rats: Rearing of Experimental Young by Stock Females	Male Rats: Testicular Degeneration	Male Rats: Sterility	Reference
A. Milk diet									
Milk + 0·5 mg. Cu + 1·0 mg. Fe/100 ml.	None							+	Waddell (1931)
,, ,, Cu + Fe	None							None in 1st or 2nd generation	Keil and Nelson (1936)
,, + 0·5 mg. Cu + 1·0 mg. Fe/100 ml.	None		Irregular	−					Waddell *et al.* (1931)
Ditto.	0·5 mg. per 100 ml.		Improved						
,, + 0·16 mg. Cu + 0·5 mg. Fe per rat daily	None			−					Krauss (1931)
,, + 0·5 mg. Cu + 1·0 mg. Fe/100 ml.	None		Normal						Orent and McCollum (1932)
Ditto.	0·5 mg. per 100 ml.		Normal						
,, + 0·5 mg. Cu + 1·0 mg. Fe/100 ml.	None		Irregular						Skinner *et al.* (1932)
Ditto.	1·0 mg. per 100 ml.		Improved but not normal until sucrose added						
,, + 0·05 mg. Cu + 0·5 mg. Fe per rat daily	None			−					Keil *et al.* (1934)
Ditto.	0·37 mg. daily			+ (2 generations)					
,, + 0·8 mg. Cu + 5·0 mg. Fe + KI/100 ml.		*0·022 mg.		−	+	−			Daniels and Everson (1935)
Ditto.	1·5 mg. per 100 ml.	1·202 mg.		+					
,, + 0·1 mg. Cu + 1·0 mg. Fe/100 ml.	None		Irregular				+	+	†Boyer *et al.* (1942)
B. Solid diet									
(*a*) Casein, butterfat, maize starch, yeast extract, viosterol, salt mixture	None		(Normal controls, no data)						Orent and McCollum (1931)
(*b*) Diet (*a*) with all Mn extracted ("Mn-free" diet)	None		Normal	−	−	−	+	+	
(*c*) Ditto.	0·005 per cent.		Normal	Marginal			Normal		
(*d*) Ditto.	0·05 per cent.		Normal	+					
Whole milk powder, casein, cottonseed oil, sucrose, salts, vitamins. Low Mn diet		0·003 mg.	Normal	+ (1·5 per cent. reared)	+	−	Normal in 1st generation (in 2nd generation)		Shils and McCollum (1943)
Ditto.	100 mg./litre of drinking water	Not stated	Normal	+ (56 per cent. reared)					

* If this figure is correct the other minerals must have been contaminated with Mn, since the milk contained only 0·003 mg. Mn per 100 ml.

† The results of Boyer *et al.* (1942) refer to the fertility of male and female offspring of female rats given the low Mn diet for the last few days of gestation and during lactation.

born to mothers receiving 5 mg. Fe daily as a supplement to the dried whole milk diet. The Fe content of second litter pups produced by does on the unsupplemented diet was only half that of first litter pups from the same does. None of the does on the milk diet succeeded in rearing second litter pups to weaning, probably because of Mn depletion, but the results confirm those of earlier workers (see Davidson and Leitch, 1934) that the presence of a normal reserve of Fe at birth depends on an adequate supply in the mothers' diet.

Comparison of Different Levels of Copper in the Diet

Breeding Performance

Rats

Cunningham (1931) found that young female and male rats grew well and throve on a diet of mixed foods selected so as to give a daily intake of 0·011 mg. of Cu per rat. The first litters borne by the does were slightly smaller in size and in average birthweight per pup than those of a group which received an additional supplement of $CuSO_4$ to give a daily intake of 0·05 mg. Cu (the amount provided to control rats by a stock diet), although the number of observations was not large enough nor the differences sufficiently great for the results to be considered significant. Nor was the period of observation, which did not include the rearing of the litters, sufficiently long.

Foetal Supply of Copper

Rats

Cunningham (1931) stated that the Cu content of the liver of the rat (and of other species) was at a maximum at birth and subsequently declined, but this finding is not confirmed by Brückmann and Zondek (1940) who found that the Cu content of the rat liver rose after birth to a maximum between the 10th and the 15th day of life. Cunningham observed that the feeding of Cu to the mother caused increased storage of Cu in the newborn rat but had no effect on the storage of Fe in the foetus.

Iron and Copper Deficiency Anaemia

From early work on nutritional anaemia due to deficiency of Fe or Cu or both, reviewed by Davidson and Leitch (1934), Garry and Stiven (1936) were unable to find any evidence of a direct effect of Fe or Cu deficiency anaemia on reproduction in experimental animals. Nor has the position been clarified in recent years. It may be of interest to note that Cox and Mueller (1937), in a detailed study of the yield and composition of the milk of stock rats, found concentrations of Fe and Cu 10 times as great as in cow's milk.

Restricted Supply of Manganese

oestrous cycle, testicular degeneration and rearing of young

Rats

The conflicting results obtained by different workers with Mn-deficient diets can be seen from Table XIV, but there is sufficient agreement to warrant the statement that diets of purified foods are not readily obtainable so low in Mn as to interfere with normal oestrus, although low enough to cause male sterility and congenital debility in the young of does reared on such diets. Shils and McCollum (1943) attempted to explain the lack of unanimity in the findings on the effect of Mn deficiency on the oestrous cycle and the ability of mothers on a Mn-deficient diet to suckle young stock rats, on the basis of degrees of Mn deficiency due to differences in the Mn content of the diets used. The discrepancies may be partly explained by the level of Mn reserves in the rats when given the experimental diet (Boyer *et al.*, 1942); in the investigations of Shils and McCollum (1943) stock female rats transferred to a low-Mn

diet during the last week of pregnancy succeeded in rearing their young, which were all of good weight and appearance at 21 days of age. The picture of Mn deficiency on a diet of liquid milk, Fe and Cu is complicated by deficiency of energy (Skinner *et al.*, 1932), and possibly by lack of some other substance essential for lactation (Richardson and Hogan, 1940), but conflicting results have been obtained even within the milk group of experiments. Differences in the Ca and P contents of the diets may contribute to the different results obtained with milk and solid diets and with different solid diets, since the work of Shils and McCollum (1943) indicates that the Ca and P contents of the diet affect the utilisation of Mn by the growing rat.

The role of Mn in the nutrition of the female rat during gestation and lactation is not understood. Shils and McCollum (1943) showed that the effects of Mn deficiency in female rats can be reversed by administration of Mn even after 428 days on the deficient diet. They found abnormal bone development in the offspring of Mn-deficient does, an observation in agreement with the findings of Barnes *et al.* (1941), who used a low-Mn milk diet. They also found that the young of the few mothers on Mn-deficient diets that succeeded in rearing their young exhibited ataxia, inco-ordination and loss of equilibrium. Anaemia apparently does not occur in rats on diets deficient only in Mn.

Mice

The effect of Mn deficiency on reproduction and lactation has been studied also in mice. Kemmerer *et al.* (1931) observed that mice reared on a diet of whole milk supplemented with 0·15 mg. Fe and 0·01 mg. Cu per mouse daily did not ovulate normally. Mice reared on this diet with a daily supplement of 0·01 mg. Mn had normal oestrous cycles. Shils and McCollum (1943) reared a group of mice from weaning on a solid Mn-free diet and another group on the same diet with a supplement of Mn in the drinking water. In the unsupplemented group 4 female mice had a total of 10 litters with 61 young, of which all but 6 died within 48 hours and none live more than 3 days. In the supplemented group 5 had 14 litters with 95 young, of which 33 were weaned.

Rabbits

Smith *et al.* (1944) reported the occurrence of testicular degeneration and reduction in the size of ovaries and uteri in rabbits reared on a diet of milk powder supplemented with 2 mg. Fe, 0·2 mg. Cu and 0·1 mg. pyridoxine daily, but not in controls receiving a daily supplement of 5 mg. Mn.

Foetal Supply of Manganese

Rats

Skinner *et al.* (1931) compared the Mn content of young rats from mothers on a stock diet low in Mn with that of young from mothers which received a daily supplement of 5 mg. Mn. The percentage Mn content of the young was highest at birth and was 40 per cent. higher in the supplemented group than in the unsupplemented, but there was no appreciable difference between the groups at 12 days of age. They concluded that addition of Mn to the mothers' diet resulted in an increased placental transfer of Mn but no significant increase in the transfer of Mn through the milk. Orent and McCollum (1931) were unable to detect the presence of Mn in the newborn young of rats raised on an Mn-free diet, although it was present in the young of mothers which had received Mn in the diet. Daniels and Everson (1935) showed that the offspring of rats on a diet of milk, Cu and Fe supplemented with 1·5 mg. Mn per 100 ml., contained at birth about 4 times the amount of Mn found in the offspring of rats which had received the milk, Cu, Fe diet without the Mn supplement.

Requirement of Manganese

Rats

The nearest approach to an estimate of the Mn requirement of the rat for reproduction and rearing of the young is the finding of Orent and McCollum (1931) that inclusion of Mn

at a level of 0·005 per cent. in a purified synthetic diet gives marginal performance, and at a level of 0·05 per cent. successful performance. If the average food consumption of the rat during gestation and lactation is assumed to be approximately 25 g. daily, a level of 0·005 per cent. of Mn would supply approximately 1·25 mg. Mn per rat daily, which is of the same order as the amounts supplied by Daniels and Everson's supplemented milk diet (1·202 mg. Mn) and half as much again as their stock diet (0·816 mg. Mn), which gave good results. Richardson and Hogan (1940) estimate that their stock diet, which permitted good reproductive performance, would supply only 0·35 mg. Mn per rat daily, compared with 1·02 mg. Mn per rat in their diet of fresh and dried milk supplemented with Fe, Cu and Mn, which did not give such good reproductive performance as the stock ration. It appears as if there are still uncontrolled variables to be investigated.

To sum up, it may be said that published observations on the effect of Fe, Cu and Mn in the diet on the reproductive performance of laboratory animals are too inadequate or too inconsistent to permit of any generalisation about the requirements of these elements. The most that can be said is that an intake of 0·05 mg. of Cu daily is probably adequate and that a synthetic diet should have 1 mg. or more of Mn added per 1at daily ; a diet of natural foods with less Mn than this may be adequate.

SODIUM AND CHLORINE

Restricted Supply of Sodium

sexual maturity, oestrous cycle, male fertility

Rats

Orent-Keiles *et al.* (1937) compared the effect on reproduction of rats of a purified diet containing 0·002 per cent. of Na with that of the same diet in which 1·4 per cent. of $NaHCO_3$ (0·38 per cent. of Na) was included. Sexual maturity was delayed and oestrous rhythm in female rats was affected on the low-Na diet, although male rats remained fertile for at least 75 to 96 days on the restricted Na intake. During about 10 weeks after the attainment of sexual maturity, Na-depleted female rats were caged for 10 to 12 hours out of each 24 hours with stock colony male rats, during which time the rats had no access to food. For the remainder of the 24 hours the rats were returned to their individual cages and given their previous diets. Although the does were under observation during the day throughout the experimental period, copulation was not observed. Post mortem examination at the end of 10 weeks showed no sign of pregnancy in 10 out of 12. In one rat 1 foetus was found and in another 5 foetuses. These workers criticise previous reports on the effects of low-Na diets on the ground that the diets were in other respects inadequate.

Sodium Content of Stock Diet

Rats

Cowie and Folley (1947) report the Na content of their stock diet as 0·44 per cent. of Na_2O on a moisture-free basis.

Comparison of Different Levels of Sodium Chloride in the Diet

Rats

In the Columbia University laboratories it has been customary to include NaCl at a level of 2 per cent. of the wheat in the stock diets of whole wheat and dried whole milk. Campbell (1946) has recently reported breeding records to 1 year of age of female rats maintained on a whole wheat, dried whole milk diet with NaCl incorporated at levels of 1·32, 2·59 and 5·06 per cent. of the diet. There appeared to be little difference in the age at which the females matured ; a larger number of young were born and reared on the diets containing the higher

levels of NaCl but the average weight of the young was significantly less at the 5 per cent. level of NaCl than at the other levels. There were indications of kidney damage in the does at the 5 per cent. level. Campbell states that previous unpublished tests with diets in which the amount of NaCl incorporated ranged from 0 to 9·09 per cent. showed no difference in breeding record until the highest level of NaCl was reached.

Barelare and Richter (1938) studied the NaCl intake of pregnant rats by the method of self-selection. They found an average daily intake of 69 mg. in the 10 days before conception, 147 mg. in the first 10 days of gestation, 219 mg. in the second 10 days and 105 mg. in the first 10 days *post partum*.

Foetal Supply of Chlorine

The Cl requirement of the rat during the reproductive period does not appear to have been studied, but Winter (1934) showed that the total Cl content of the newborn rat is about 200 to 250 mg. per 100 g. bodyweight, falling steeply during the first and second weeks of life and more slowly up to the end of the first month. Rats

POTASSIUM

Restricted Supply

Heppel and Schmidt (1938) found that when young rats were maintained on a purified diet supplemented with minerals and vitamins but containing only 0·015 per cent. of K, food consumption fell from 8 to 5 g. per head daily. There was very little liveweight increase, and death occurred in from 4 to 8 weeks, depending on the age of the animal when it was given the deficient diet. When the K intake of rats that had been maintained on the deficient diet for 8 weeks was increased to 0·58 per cent. of the diet, normal growth was resumed and the animals reproduced in a normal manner. Rats reared on this supplemented diet showed a weekly liveweight increment of 20 to 30 g. Rats

SEXUAL MATURITY AND OESTROUS CYCLE

Orent-Keiles and McCollum (1941), using a diet containing 0·01 per cent. of K, also found that food consumption in young rats decreased from 8 to 5 g. daily, but although weight increase was subnormal, loss of weight did not occur and the length of life did not appear to be reduced. In female rats sexual maturity was delayed and oestrus was irregular or absent. Rats

SPERMATOGENESIS

When male rats on Orent-Keiles and McCollum's low-K diet were mated with normal stock females pregnancy did not result. Spermatozoa were defective in number, form and motility. Rats

Male and female control rats on the same diet supplemented with K to give a content of 0·44 per cent. in the diet reproduced normally and 2 generations were reared before mating was discontinued.

Histological examination of the reproductive tissues of rats in a paired feeding test showed pathological changes in both K-deficient and control animals; in the groups fed to appetite only the K-deficient rats showed these changes. Infertility in rats on the low-K diet was therefore attributable to partial inanition rather than lack of K. (Orent-Keiles and McCollum, 1941; Follis *et al.*, 1942.)

Potassium Balances

PREGNANCY AND LACTATION

Rats

Heppel and Schmidt (1938) studied the effects on mature female rats of purified diets with different levels of K given during pregnancy and lactation. Balance experiments showed that when the K content of the diet was 0·140 per cent. the retention of K during pregnancy usually exceeded the amount of K present in the foetus at birth, but at the end of lactation all the mothers had lost weight and an amount of K which could not be correlated with the loss in weight. Many of the rats failed to lactate but this was attributed to inadequate intake of a water-soluble factor. Rats on a diet of natural foods containing 0·503 per cent. of K showed a positive K balance at the end of lactation, but the effect of the increased K content on lactation cannot be separated from the possible effects of increased amounts of other essentials in this diet. Rats maintained during pregnancy on a purified diet containing 0·011 per cent. of K did not gain in weight during pregnancy, although they succeeded in producing young that weighed from 3·7 to 5·0 g. each at birth and which were eaten shortly after birth. Rats maintained on an adequate diet during pregnancy were divided into two groups at parturition and given diets containing 0·183 and 0·018 per cent. of K, respectively. Rats in the latter group were found to be in negative and those in the former in positive K balance. Those on the low-K diet lost weight, while the others gained weight, and the offspring of the K-deficient mothers at weaning were much lighter in weight than the young of mothers on the higher level of K, but had approximately the same percentage content of K.

COMPARISON OF DIFFERENT LEVELS OF POTASSIUM IN THE DIET

Breeding Performance

Rats

The above results show that rats which survive when reared on a diet containing 0·01 per cent. of K are unable to reproduce, although paired feeding tests indicate that the changes in the sex organs are the result of reduced food intake rather than of K deficiency *per se* (Orent-Keiles and McCollum, 1941; Follis *et al.*, 1942). A diet containing 0·44 per cent. of K is adequate for reproduction over at least two generations (Orent-Keiles and McCollum, 1941) although the actual requirements during pregnancy and lactation may be met by smaller amounts of the order of 0·14 to 0·18 per cent. (Heppel and Schmidt, 1938.)

Potassium Content of Stock Diet

Rats

Cowie and Folley (1947) report the K content of their stock diet as 1·06 per cent. of K_2O on a moisture-free basis.

ALUMINIUM

Rats

Hove *et al.* (1938) were unable to demonstrate any effect on the oestrous cycle or reproduction of rats on a mineralised milk diet providing 1 μg. Al per day when a daily supplement of 10 or 30 μg. Al as its sulphate was given.

BORON

Rats

Orent-Keiles (1940) and Teresi *et al.* (1944) found no evidence that a supplement of B added to basal diets containing 16·3 and 15·5 μg. B per 100 g. had any effect on weight increase, reproduction or rearing of young rats, and concluded that if B is needed for reproduction and lactation the need is met by diets with this low level.

FLUORINE

The effect of F-free diets on fertility, reproduction and lactation in laboratory animals has not been tested. The small amount present in milk, 0·1 to 0·2 p.p.m., or 1·6 p.p.m. on a dry matter basis, can apparently supply any need the rat may have for F during growth and reproduction (Evans and Phillips, 1939). Rats

Excess of Fluorine

The literature relating to the toxic effects of F has been reviewed by Peirce (1939), who points out that the amount of F necessary to produce toxic symptoms depends on a variety of circumstances, including the nature of the F compound, the method of administration and the amounts of other dietary constituents.

Lamb *et al.* (1933) and Phillips *et al.* (1933) have studied the effects of administration of NaF on fertility and reproduction in rats. When the daily intake of F was increased to about 20 mg. per kg. bodyweight by administration of NaF, the weight increase of female rats was adversely affected and the birthweights and weaning weights of their young were reduced. The oestrous cycle was unaffected until the dosage of F reached the level where inanition occurred as a result of reduced food intake. Intakes of 25 to 30 mg. F as NaF resulted in complete cessation of oestrus. A similar effect could be produced by restricting the food intake without increasing the F content of the diet. Phillips *et al.* (1933) concluded that the suppression of oestrus on a high intake of F was probably due to inanition. Similarly, the low weaning weights were attributed to the reduced food intake during lactation. Rats

Smith and Leverton (1934*b*), who studied the effect of increasing the F content of the diet of young rats by adding fluorides, also found that interference with reproduction occurred only at those levels of F intake which caused stunting of the female. Four generations were reared on diets containing 0·0113 or 0·0226 per cent. of F, but in the latter case, although the percentage of fertile matings and the number of young born were not reduced, the percentage of young weaned and their average weaning weights were. At a level of 0·0452 per cent. of F the female rats did not reproduce, but after 6 months on this diet they were able to reproduce when transferred to a stock diet.

Transfer of Fluorine from Mother to Young

The F content of the mother's diet affects the F content of the young rats at birth and weaning (Reid and Cheng, 1937; Evans and Phillips, 1939). Schuck (1938), who found evidence of fluorosis before weaning in the teeth of young rats whose mothers had reproduced on a diet containing 0·1 per cent. of NaF, considered the transfer of fluorides through the milk to be more important than transference through the placenta but, according to the findings of Evans and Phillips (1939), the increase in F content of the young which results from supplementing the mother's diet of mineralised milk with F occurs mainly during gestation. Rats

SELENIUM

Excess of Selenium

Franke and Potter (1936) found that if weanling rats were given a diet containing 24·6 p.p.m. Se supplied as seleniferous wheat they died within 21 days. If the seleniferous wheat was not introduced until the rats were 42 days old they survived, but litters were not obtained when the male and female rats were mated together or with stock rats. In some of the females Rats

the reproductive organs were found to be underdeveloped. If the transfer to the toxic diet was delayed to 186 days of age, the rats still failed to mate successfully with one another, although fertile matings occurred when male rats were mated with stock does and occasionally when does were mated with stock male rats. In no case did an experimental doe succeed in rearing her young. Records of food intake showed a marked decrease in consumption of the diet containing the seleniferous wheat.

Munsell *et al.* (1936) found that the toxic effect of diets containing Se was proportional to their Se content. Diets containing 1·5 p.p.m. Se in the form of seleniferous wheat did not affect weight increase or reproduction in rats, but with higher levels of Se weight increase and reproduction were progressively affected until at 7·5 or 8·7 p.p.m. Se, rats were stunted, only 33·3 per cent. of matings were fertile, only a few young were born and none was reared. Records of food consumption showed that ingestion of Se caused a reduction of food intake. At the level of 7·5 p.p.m. Se in the diet the food consumed supplied 0·25 mg. Se per 100 g. bodyweight daily.

The literature on Se poisoning has recently been reviewed by Moxon and Rhian (1943). The main symptoms of poisoning in rats caused by feeding seleniferous grains have been reproduced by feeding inorganic Se. In the form of selenious acid, Se appears to be less toxic than when occurring naturally in wheat. The toxic symptoms are not simply secondary effects of the inanition ; but the extent to which the effects on reproduction can be attributed to inanition has not been ascertained.

Transfer of Selenium from Mother to Young

Rats

The placental transmission of Se in the rat was demonstrated by Westfall *et al.* (1938), who found that when Se was added to the diet of pregnant rats in early or mid-pregnancy, up to 14 per cent. of the mother's intake of Se was found in the newborn young.

Cats

They also showed that Se is transferred through the placenta in the cat.

ESSENTIAL FATTY ACIDS AND FAT

Restricted Supply of Fat

Rats

A deficiency disease characterised by skin and kidney lesions, caudal necrosis, retardation of liveweight increase and increased water consumption, occurring in rats on a low-fat diet, was first described by Burr and Burr (1929).

OESTROUS CYCLE

Rats

Disturbance of the oestrous rhythm in female rats on low-fat diets was reported by Burr and Burr (1929 ; 1930), Evans *et al.* (1934), Turpeinen (1938) and Ershoff (1943), but not by Quackenbush *et al.* (1942).

TESTICULAR DEGENERATION

Rats

On low-fat diets male rats show testicular degeneration (Burr and Burr, 1930 ; Evans *et al.*, 1934 ; Quackenbush *et al.*, 1942 ; Ershoff, 1943).

The condition described by these investigators did not appear to be due to lack of vitamin A or E, since these vitamins were supplied as the unsaponifiable fraction of cod liver oil and wheat germ oil or as carotene and α-tocopherol. Vitamins of the B complex were supplied as ether-extracted yeast, as a rice-bran concentrate or as synthetic compounds.

PREGNANCY

The absence of linoleic acid from the diet of pregnant rats previously depleted on a low-fat diet caused prolonged gestation, difficult labour, a high incidence of stillbirths, small litters of small average weight, and inability to suckle the young (Evans *et al.*, 1934; Maeder, 1937; Quackenbush *et al.*, 1942). Rats

Effects of Supplements of Essential Fatty Acids

OESTROUS CYCLE

Burr and Burr (1930) obtained normal ovulation by supplying a few drops of cod liver oil and wheat germ oil instead of the non-saponifiable fractions, and fertile matings resulting in the production of litters were obtained with stock male rats. A few drops of lard prevented the occurrence of sterility in male rats. The deficiency condition in young rats was prevented by administration of a few drops of fat containing unsaturated fatty acids or by methyl linoleate. Burr and Burr concluded that linoleic acid was an essential fatty acid. Rats

Turpeinen (1938) found that the number of oestrous cycles in female rats on low-fat diets was increased by daily administration of methyl linoleate, methyl arachidonate and linoleyl alcohol. The highest number of cycles was obtained with administration of 100 mg. methyl linoleate, the dose which gave maximum growth response.

PREGNANCY AND LACTATION

Evans *et al.* (1934) found that daily addition of a mixture of 40 mg. linoleic acid and 10 mg. oleic acid to a low-fat diet during gestation and three times these amounts from parturition caused a marked improvement in reproductive performance, but the rearing of young remained poor. The fatty acid preparation used was made from maize oil by the crystallisation and removal of its insoluble lithium salts from 80 per cent. alcohol. The basal diet consisted of fat-free casein 24·0, sucrose 72·1 and salt mixture 3·9 per cent., supplemented by vitamins as indicated above. Rats

Results similar to those obtained with the diet low in fat and free of linoleic acid were obtained with a diet in which the sucrose was omitted, the casein increased to 38 per cent., the salt mixture to approximately 4·5 per cent. and the rest of the diet consisted of hydrogenated coconut oil 57 per cent. Addition of the mixture of linoleic acid and oleic acid to this diet enabled successful gestation to occur, but lactation was still unsatisfactory as judged by the weaning weights of the young.

Mackenzie *et al.* (1939) took the precaution of adding cystine to supplement the casein in their low-fat ration, but they gave only 25 mg. ethyl linoleate per rat daily. They were able to carry their rats through 3 gestations, but the rearing of litters was poor. Results obtained by exchanging the young of mothers in the supplemented low-fat diet for young from healthy stock mothers indicated that milk production was inadequate.

Quackenbush *et al.* (1942) suggest that at least 100 mg. of the essential fatty acids should be given daily to rats during pregnancy and lactation. They found that addition of 5 drops of ethyl linoleate daily, from the second week of gestation, to a diet consisting of fat-free casein, glucose, salts, rice-bran concentrate and synthetic vitamins gave results which compared favourably with those obtained on their stock diets. The average length of gestation was normal, the average litter size at birth was 6, and 70 per cent. of the young were weaned at 21 days, weighing on the average 40 g. It is unfortunate that some workers still quote doses in terms of drops, but on the assumption that the drop in question equalled 0·05 ml. the daily dose of ethyl linoleate was of the order of 250 mg. per rat.

Guggenheim and Jürgens (1944) gave female rats from the 13th day of pregnancy a diet consisting of glucose 78, casein 18 and salts 4 per cent., with a vitamin supplement per head daily in μg. of vitamin B_1 10, riboflavin 40, vitamin B_6 20, choline 3000, nicotinic acid 500, pantothenic acid 100, vitamin D 5, β-carotene 40 and *dl*-α-tocopherol acetate 400. Other rats received a supplement of linoleic acid, the amount of which is not stated. In the supplemented group the mothers remained normal. The litters appeared normal at birth but within 5 days caudal necrosis developed; later the young lost weight and died within 14 to 21 days. No other skin change was observed. The blood picture was abnormal. Administration of 50 mg. linoleic acid daily to the mothers when the young were showing these symptoms resulted in an improvement in the condition of the young. Similar results were obtained with a diet containing 9 per cent. of hardened coconut oil and administration of linoleic acid to the young was curative. Bernard and Bodur (1946) determined the amounts of fatty acids in the bodies of does and of litters at birth when the fat-free diet was given from the 13th day of pregnancy. The liquid acids were brominated and the amount of *cis, cis*-9 : 12 linoleic acid tetrabromide estimated. Both the adult does and young in a control group on a normal diet had a higher total fatty acid percentage than the corresponding animals on the fat-free diet. The control does gave much more of the tetrabromide than the does on the fat-free diet. The young in the control group gave appreciable amounts of the tetrabromide but the young of the does on the fat-free diet yielded none. Thus although the does on the fat-free diet had in their bodies *cis, cis*-9 : 12 linoleic acid, they appeared to be unable to pass on this essential acid to their young. The young showed the characteristic tail lesions described by Guggenheim and Jürgens.

Evidence is accumulating from investigations with young rats that vitamin B_6 exerts a sparing action on linoleic acid but that both are essential for the rat. The requirements of the rat for linoleic acid during the reproductive period will probably depend on the vitamin B_6 content of the diet, at any rate at lower levels of this vitamin. Exact quantitative estimates of linoleic acid requirements for reproduction and lactation for any level of vitamin B_6 are, however, lacking.

The position is further complicated by evidence that α-tocopherol also exerts a sparing action on linoleic acid (Hove and Harris, 1946).

And finally, since certain other unsaturated fatty acids such as arachidonic and linolenic can replace linoleic acid in the diet of the growing rat and can increase the number of oestrous cycles of female rats on a low-fat diet (Turpeinen, 1938), such acids may also be able to replace linoleic acid in facilitating reproduction on fat-free diets.

Comparison of Different Levels of Fat in the Diet

LACTATION

Rats

That fat *per se* in the diet exerts a beneficial effect on the ability of does to rear their young, by virtue of some factor other than its content of unsaturated fatty acids, is indicated by the observations of some but not of all workers.

Evans *et al.* (1934) found that although daily addition to a fat-free diet of a mixture of 40 mg. linoleic acid and 10 mg. oleic acid per rat during gestation, and three times these amounts from parturition, improved reproductive performance, lactation remained poor. Inclusion of 25 per cent. of lard or butterfat brought the lactation performance up to that of rats on a stock ration. Evans *et al.* concluded that in addition to essential unsaturated fatty acids appreciable amounts of certain fats are necessary for normal lactation. Reproductive performance of rats receiving added fat was also improved as indicated by the average birth-weight of young.

Mackenzie *et al.* (1939) also reported poor lactation in rats on a fat-free diet supplemented with 25 mg. ethyl linoleate per rat daily. On the other hand, Quackenbush *et al.* (1942), with a daily dose of 5 drops of linoleic acid (250 mg. ?), obtained on a low-fat diet reproduction and lactation equal to that of their stock rats. The basal diet was very carefully treated to remove fat, and the total daily lipid intake from this source was only 3·1 mg., so the results did not suggest any need for substantial amounts of fat *per se* in the diet. Richter and Barelare (1938) showed, however, that rats given access to casein, dried yeast, sucrose, olive oil, salts, cod liver oil and wheat germ oil selected a diet in which the percentage of calories from fat increased from 52·6 during the last 5 days before mating to 54·3 during the last 5 days of pregnancy and 65·0 during the last 5 days of lactation.

Maynard and Rasmussen (1942) found, in paired feeding experiments, that diets containing 9·2 and 18·5 per cent. of fat resulted in much better lactation performance in rats than diets containing 4·5 and 0·3 per cent. of fat, when fed on an equicaloric basis from parturition. The improved lactation performance, as judged by the weight and composition of the young at 17 days, was obtained in part at the expense of the mother's reserves, as was indicated by the weight lost by the does. When the high-fat (18·3 per cent.) diet was given to appetite the does gained weight and the young were heavier at 17 days of age than those of does on the high-fat diet whose intake of food was limited according to the appetite of the group on the low-fat diet. In a subsequent report (Loosli *et al.*, 1944) results are presented from which it is concluded that the addition of fully hydrogenated coconut oil to a low-fat diet had not the stimulating effect of maize oil on lactation, and, further, that although the low-fat diet with added hydrogenated coconut oil would be lacking in unsaturated fatty acids supplied by the maize oil, deficiency of linoleic acid was not the limiting factor, since administration of 125 mg. ethyl linoleate daily to the lactating does on the low-fat diet had no beneficial effect, nor had direct administration to the young. It appeared, therefore, that some property other than its linoleic acid content distinguished one fat from another in its effect on lactation of rats not previously depleted of linoleic acid. All diets contained 0·2 per cent. of cystine. The increased weight gain in the young of mothers on high-fat diets was largely an increase in fat (Maynard and Rasmussen, 1942 ; Loosli *et al.*, 1944). Loosli *et al.* found that in their paired feeding experiment most of the does lost weight during lactatior but, unlike Maynard and Rasmussen, they were unable to correlate weight lost during lactation with the type of diet fed.

Deuel *et al.* (1944) showed that in rats maintained from weaning on diets containing 70·6 per cent. of skimmed milk powder supplemented with Mn, Cu and Fe salts and 29·4 per cent. of fat fortified with vitamins A, D and E, successful pregnancy ensued irrespective of whether the fat was butter, margarine, or maize, cottonseed, olive, peanut or soya bean oil. These fats were equally good from the standpoint of lactation. Nor was there any significant difference in the growth of the young weaned on to these diets (Deuel *et al.*, 1945*a*).

Deuel *et al* (1947) studied the effect on reproductive performance of rats fed from weaning on purified fat-free diets supplemented with methyl linoleate, ethyl laurate or 5 per cent. or more of cottonseed oil. After 16 weeks on the experimental diets 8 to 10 female rats from each group were mated with male rats from the same dietary group. None of the female rats in the methyl linoleate group became pregnant. Two in the group receiving only the saturated fatty acid ester, ethyl laurate, became pregnant and reared their litters but the weaning weights of the young were very low. Of the groups receiving diets containing 5, 10, 20, 30, 40 and 50 per cent. cottonseed oil or a stock ration, the 5 per cent. fat diet gave the best results as judged by length of gestation, average number of young per litter born and reared and average weight of litter at 3 and 21 days. Only the group receiving 10 per cent. fat achieved the percentage of successful matings obtained in the stock diet group, 90 per cent.

The reason for the complete failure to mate of rats on the diet supplemented with methyl linoleate is difficult to understand in the light of earlier work. The diet contained just under 1 per cent. methyl linoleate and, on the basis of total food consumption for the 12 weeks from weaning, provided on the average about 100 mg. methyl linoleate daily. This complete lack of fertility is even more surprising when the results of another experiment with this diet are considered. In this the calorie intake of male and female rats during the 12 weeks post-weaning was reduced to about one-third and the percentage content of linoleate approximately trebled so that the net effect must have been to maintain the intake of methyl linoleate at the above level. When the rats subsequently were given the diet containing 1 per cent. of methyl linoleate to appetite, 67 per cent. of matings were fertile (Scheer, Soule *et al.*, 1947).

Excess of Fat

Rats

Vinson and Cerecedo (1944) found that when rats were given a purified diet and were offered lard to appetite large quantities were consumed. The harmful effect on rearing of young which resulted was attributed to the reduced intake of other components of the diet, not to the high fat intake *per se*. Similarly Nelson and Evans (1947*a*) observed that the percentage of young reared to weaning and the average weaning weight were lower for does reared on a diet containing 48 per cent. crisco than for does on the same diet but with only 8 per cent. crisco, the rest being replaced by an equal amount of sucrose. The loss of weight of the does during lactation on the high fat diet was very much greater than that of does on the diet with the lower level of fat. Nelson and Evans point out that in their experiment the fat was not given separately to appetite but was incorporated in the diet. The food intake was lower on the high-fat diet than on the diet with less fat. The deleterious effect of the high-fat ration was evident also in short term experiments in which the does were not transferred from the stock diet until parturition (Nelson and Evans, 1947*b*).

In the short term experiments levels of fat ranging from 48 to 0 per cent. were compared. The percentage of sucrose in the ration was adjusted on a weight for weight basis to changes in the fat content so that together they represented 72 per cent. of the diet. The percentages of protein and salts remained at 24 and 4, respectively. The results are shown in Table XV.

TABLE XV

LACTATION IN RATS ON PURIFIED DIETS OF VARYING FAT CONTENT (SHORT-TERM STUDIES)

Fat in Diet	No. of Litters	No. of Young Born	Average Wt. 1 Day	Average Wt. 21 Days	Weaned 21 Days	Mothers Weaning 5 or 6 Young	
						No.	Weight Change of Mother during Lactation
Per Cent.			g.	g.	Per cent.		g.
0*	10	30 ♂	6·2	41	97	10	−10
		30 ♀	5·8	39			(+5 to −24)
8	20	60 ♂	6·2	44	93	18	−25
		59 ♀	5·9	43			(−5 to −54)
24	10	30 ♂	6·1	33	90	9	−40
		30 ♀	5·9	33			(−9 to −55)
48	24	75 ♂	6·3	35	96	23	−63
		69 ♀	5·9	34			(−11 to −90)

* One ml. maize oil was given weekly to each litter, in addition to the fat-soluble vitamins, to insure an adequate intake of the essential unsaturated fatty acids.

Litters were reduced to 6 at birth. The percentage of young weaned was greatest and the loss in weight of the mothers least in the group in which all the fat was replaced by sucrose.

Since food intake was less at the higher levels of fat a subsidiary experiment was made with the diets containing 8 and 48 per cent. of fat in which the vitamin intake was doubled. This resulted in a slight improvement in the weaning weights of the young but did not affect the weight lost by the does. The beneficial effects of reduction of fat were therefore not attributed to increased consumption of vitamins.

The most important effect of the increased fat content in the investigation of Nelson and Evans was the increased loss in weight of the does during lactation. In none of the groups did the does gain in weight. On the other hand Loosli *et al.* (1944) observed that their lactating does lost weight irrespective of the fat content of the diet. Maynard and Rasmussen (1942) contrary to both these findings observed that does on a diet containing 18·5 per cent. of fat gained weight when the diet was fed to appetite, while those on a low-fat diet, 0·3 per cent., lost weight. It should be noted that Nelson and Evans have recorded elsewhere (Nelson and Evans, 1947*c*) an average increase in weight of 20 g. during lactation in does on a stock diet previously reported to contain 11 per cent. of fat (Nelson and Evans, 1947*a*).

Further work is needed to settle this question.

VITAMIN A AND CAROTENE

Restricted Supply

CORNIFICATION OF THE VAGINA

Rats

The discovery of Evans and Bishop (1922) that a diet low in vitamin A causes persistent cornification of the vagina in female rats has been confirmed by many investigators. The condition appears to be a specific sign of vitamin A deficiency in the rat, and has been used as a criterion in the biological estimation of vitamin A. Goss and Guilbert (1939) observed that when young female rats whose mothers had received a vitamin A-free diet during the last 10 days of lactation were weaned to this diet at 21 days, they showed persistent cornification of the vagina for 5 successive days at an average age of 53 days. When cod liver oil or carotene was given at different levels to groups of these rats it was found that the minimum daily allowance required to prevent persistent cornification was between 3·8 and 4·6 μg. or 18 to 22 I.U. vitamin A as reference cod liver oil, or between 15 and 20 μg. carotene, per kg. bodyweight (a mixture of 88 per cent. β- and 12 per cent. α-carotene). The basal vitamin A-free diet used in this investigation consisted of maize starch, heat-treated casein, irradiated yeast, cottonseed oil and a salt mixture. Callison and Knowles (1945) confirmed the findings of Goss and Guilbert (1939) that a daily intake of between 15 and 20 I.U. per kg. bodyweight in the form of a cod liver oil supplement to a basal vitamin A-deficient diet prevents the occurrence of persistent cornification of the vagina.

OVARIAN DEVELOPMENT AND OESTROUS CYCLE

Rats

Truscott (1947) compared the weights of ovaries of young female rats on a vitamin A-deficient diet and a stock diet. Groups of rats were killed at intervals between the ages of 43 and 77 days. The animals on the deficient diet gained weight less rapidly than stock rats and the weight of the ovaries steadily decreased while those of the stock animals steadily increased.

Regular oestrous cycles may occur in vitamin A-deficient rats showing persistent cornification of the vagina. Irregularity of the oestrous rhythm, when it occurs, appears

from the evidence of paired feeding experiments to be a secondary effect of inanition (Mason and Ellison, 1935; Kudrgashor, 1935; Richter and Barelare, 1939).

Mice

Mice reared on a diet of purified casein 31, dextrin 35, crisco 22, salts 7 and yeast 5 per cent. showed symptoms of vitamin A deficiency, including xerophthalmia, but there was no noticeable change in the female genital tract; ovaries, fallopian tubes, uterus and uterine glands appeared normal (Wolfe and Salter, 1931).

TESTICULAR DEGENERATION

Rats

Male rats on purified diets low in vitamin A, usually of casein, dextrin or starch, cottonseed oil, yeast, salts and sources of vitamins D and E, showed atrophy of the testes and sterility which was not due to lack of appetite but was attributable to deficiency of vitamin A (Sampson and Korenchevsky, 1932*a*, *b*, *c*; Sampson *et al.*, 1932; Evans, 1932; Mason, 1933; Mitzkewitsch, 1934; Moore and Mark, 1936; Küttner, 1939).

Mason (1933) used an A-deficient diet of purified casein, dextrin, crisco and salts supplemented with 0·5 g. yeast and 0·05 mg. irradiated viosterol daily. He found that the testicular degeneration could be cured by replacing half of the crisco by lard and adding cod liver oil. When cod liver oil was added without the lard the degeneration attributed to vitamin A deficiency was reversed, but degeneration attributed to deficiency of vitamin E became apparent. Rats showing signs of testicular injury due to uncomplicated vitamin A deficiency were given cod liver oil or cod liver oil and wheat germ oil. Vitamin E deficiency developed under the former treatment but not under the latter. Mason suggested that the requirement of vitamin E might increase as the germ cells of the testes became deprived of vitamin A.

Hansard and Sutton (1941) reared male rats from weaning on a vitamin A-deficient diet supplemented with 10, 20 or 30 I.U. vitamin A per kg. bodyweight. The degree of testicular degeneration observed was inversely proportional to the level of vitamin A intake. The highest level did not afford complete protection.

Mice

Wolfe and Salter (1931) found testicular degeneration in male mice on a vitamin A-deficient diet.

PREGNANCY

Rats

Mason (1935) described the occurrence of foetal death, prolonged gestation and difficult labour, frequently accompanied by excessive haemorrhage, in female rats on diets low in vitamin A although the condition was not pathognomonic of vitamin A deficiency. It did not occur in all the rats on a low intake of vitamin A and occurred in some rats receiving the minimal dose of cod liver oil necessary to prevent cornification of the vagina. When adequate amounts of vitamin A were supplied as cod liver oil during the second pregnancy, normal gestation and parturition usually ensued. Tansley (1936), Newton (1938) and Cannon (1940) produced a similar syndrome which could be prevented by administration of carotene.

In the course of an investigation of the effect of vitamin A deficiency in the maternal diet on the dental tissues of young rats, Mellanby (1941) made some observations on reproductive performance. The vitamin A-deficient diet used appeared to be adequate in other respects, since the addition of a vitamin A concentrate (the amount of A is not stated), permitted successful reproduction over 5 generations. Female rats were given the vitamin A-deficient diet at the age of 3 to 4 weeks and were mated after intervals of from 12 to 34 weeks on the diet. Rats maintained on the deficient diet for from 12 to 25 weeks before mating were able to produce and rear litters of young, although examination of the livers of mothers and young at the end of lactation showed little or no vitamin A after only 12 weeks'

depletion. After 30 weeks' depletion almost all the young were stillborn and dead foetuses were found unexpelled from the uteri. Some females after 34 weeks on the vitamin A deficient diet apparently did not become pregnant, in spite of receiving adequate supplies of vitamin E as wheat germ oil. When synthetic tocopherol acetate was used instead of wheat germ oil as source of vitamin E the rats were adversely affected by deficiency of vitamin A in about half the time, while those receiving vitamin A developed and reproduced normally. The difference was attributed to small amounts of vitamin A present in the wheat germ oil.

Warkany and Schraffenberger (1944*b*) raised female rats on a diet containing very small amounts of carotene until they reached a weight of 150 to 160 g. and a regular oestrous cycle was established. The rats were then given a purified vitamin A-free diet and mated with stock male rats. The purified diet consisted of sucrose 68, vitamin-free casein 18, vegetable oil 10, salt mixture 4 per cent., supplemented in mg. per 100 g. of diet with vitamin B_1 hydrochloride 0·8, vitamin B_6 hydrochloride 0·8, riboflavin 0·8, Ca pantothenate 1, nicotinamide 10, and choline chloride 100. This diet was supplemented every 10th day by vitamins D, E and K in olive oil. Twelve of the 20 females became pregnant but of these only 3 carried their litters to term or near it; the others resorbed their embryos. The 3 litters obtained by caesarean section consisted of 19 dead young all of which had abnormal eyes. In 14 of the young the eyes were "open." The abnormalities did not occur in the young of a control group of females given a supplement of vitamin A. These observations were confirmed in a subsequent study of a larger group (Warkany and Schraffenberger, 1946). Of 90 female rats so treated 60 died before the 14th day of gestation or resorbed their embryos completely, 7 carried their litters to term and in the remainder pregnancy broke down at some stage. Examination of the embryos showed that 28 out of 39 full term young had the external eye abormally open. Histological abnormalities were observed. The findings of Warkany and Schraffenberger were confirmed by Jackson and Kinsey (1946) using the same diets and technique. Of 57 female rats bred on the vitamin A-free diet 9 died, and to prevent further deaths a dose of 20 I.U. vitamin A was given every 10 days. Only 4 rats carried their foetuses to an advanced stage of development. These foetuses showed the histological eye abnormalities observed by Warkany and Schraffenberger but not the open eye.

Wilson and Warkany (1947) studied the occurrence of epithelial keratinisation in embryos and newborn young of female rats on a vitamin A-deficient diet. The earliest signs of metaplastic keratinisation were seen in the genito-urinary tract of the foetus at the 18th day of gestation. Only epithelial structures related to the genito-urinary tract were affected.

Guinea-pigs

According to Manzi (1935), larger amounts of carotene were required by pregnant guineapigs on a vitamin A-free diet to bring pregnancy to a successful conclusion than were required for maintenance by non-pregnant animals on the same diet. Bentley and Morgan (1945) recently reported that reproduction in guineapigs was better with vitamin A than with carotene; abortions were fewer, survival of mothers and litters, and liver storage of vitamin A were greater.

Comparison of Different Levels of Carotene and Vitamin A in the Diet

Record of Performance

Rats

Browman (1936) has described an experiment in which 4-week-old female rats were twice depleted* of vitamin A on a basal diet low in vitamin A containing 7 per cent. of wesson oil (cottonseed oil as well as hydrogenated cottonseed oil. The rats were then allocated to groups and given the basal diet supplemented with 0, 1·5, 3·0, 4·5, 6·0 and 9·0 µg. β carotene

* The meaning of the term "twice depleted" is not explained.

in wesson oil daily and were mated with stock males at 100 days of age. The rats that received no supplement of carotene failed to become pregnant. In the remaining groups reproductive success as indicated by the number and birthweight of live young produced and the number of young reared increased with each increment of carotene. The group receiving 9·0 μg. carotene produced litters of approximately the same average size and birthweight as a group given a stock ration after being twice depleted, but the latter group was more successful in rearing its young. The performance of all of the groups was inferior to that of control rats given the basal diet supplemented with 10 μg. carotene at 28 days of age without previous depletion. Even in this control group, however, only 72 per cent. of the young were weaned compared with 100 per cent. in another control group of undepleted rats on a stock diet. The experiment was not continued beyond the rearing of one litter per female. It is stated, although details are not given, that rats maintained on diets containing high percentages of cottonseed meal and low in vitamin A have reproductive performances approaching normal.

No gross abnormality of eye, limb or other organ was observed by Browman in the young at the lower levels of carotene intake. This is in agreement with the observations of Cannon (1940), who was unable to produce congenital abnormalities in the young by restricting the maternal intake of vitamin A.

Jackson and Kinsey (1946) gave daily supplements of 2, 3, 5 or 10 I.U. vitamin A to female rats which had failed to mate or had resorbed their embryos in their first experiment. A daily dose of 10 I.U. enabled the mothers to produce live young with no eye abnormality. At the lower levels of dosage eye abnormalities occurred, the degree of abnormality being related inversely to the daily intake of vitamin A. Jackson and Kinsey, by manipulation of the vitamin A intake and the degree of depletion of their rats, attempted to produce live young with eye abnormalities but were unsuccessful. They concluded that although some defective foetuses may be carried to term the amount of vitamin A required for live birth seems to be beyond the range of that associated with the production of ocular abnormalities. Jackson and Kinsey established also that foetal eye abnormalities were associated with serum vitamin A levels of less than 12 I.U. per 100 ml. in maternal blood (normal 75 I.U. per 100 ml.).

Fraps (1947) studied the effect of the quantity of carotene in the ration of albino rats on fertility, reproduction and rearing of the young. Groups of 10 female and 4 male rats were given from weaning a basal diet of maizemeal 1200, cottonseed meal 300, powdered skimmed milk 500, wesson oil 100, yeast 100, irradiated yeast 10 and salt mixture 21·5 parts by weight, supplemented with alfalfa meal as source of carotene. Females were mated with males from the same group. The young rats reared by does on the experimental diets were given a vitamin A-free diet from weaning and the time required for depletion of vitamin A was measured. The results are shown in Table XVI.

Young rats suitable for vitamin A assays should weigh 40 to 50 g. at 21 to 28 days of age, should become depleted on a vitamin A-free ration in 45 days or less and weigh 70 to 130 g. On the basis of these criteria the level of 4 p.p.m. carotene was considered to be most suitable for the production of rats for vitamin A assay work on the ration used.

The results suggest a slight superiority of the level of 8·0 p.p.m. carotene for reproduction and rearing of young over the lower levels, so that the optimum level is greater than 4 p.p.m. The low percentage of young weaned in the group receiving 4·0 p.p.m. carotene was due to cannibalism of the mothers.

Sherman *et al.* (1945) recently stated that their basal diet A used in studies of Ca and P requirements (see Table V.) contains approximately 3 I.U. of vitamin A per g. or 0·8 I.U.

TABLE XVI

EFFECT OF QUANTITIES OF CAROTENE ON PRODUCTION AND QUALITY OF YOUNG RATS

Amounts of Carotene, p.p.m.	0·5	1·0	2·0	4·0	8·0
Daily intake of carotene, γ, per female	3·6	6·8	22·4	38·2	86·1
Number of litters	24	27	31	33	36
Total number of young	154	212	216	244	251
Percentage born alive	68	80	90	90	96
Percentage weaned of those born alive	52	69	80	63	81
Percentage weighing less than 40 g. when weaned	67	28	10	0	0
Percentage weighing 40-50 g. when weaned	33	72	90	96	94
Percentage weighing more than 50 g. when weaned	0	0	0	4	6
Percentage of those weaned suitable to place on test diets after depletion	23	60	81	90	87

per Calorie, and rats have thriven for 58 generations on it. They also present evidence stated to be the weighted average results of this and previous studies by Batchelder (1934) and Sherman and Campbell (1937) to show that the reproductive span in female rats was progressively increased by doubling and trebling the intake of vitamin A, the diets being otherwise identical. Sherman *et al.* (1945) do not state how the increase in vitamin A content was contrived. Reference to the earlier investigations quoted indicates that the increase in vitamin A content was obtained by increasing the proportion of butterfat in the diet (see also page 35). Batchelder (1934) used not diet A but a modification of diet B consisting of two-thirds whole wheat and one-third dried skimmed milk plus an amount of fat equivalent to the amount in the original diet B where dried whole milk was used. The variation in the vitamin A content was obtained by using varying proportions of crisco (hydrogenated cottonseed oil) to butterfat. Reproduction did not take place when all the fat was supplied as crisco (8 per cent.) but each successive increment of butterfat from 0·5 to 8 per cent. in the diet resulted in improved reproduction and rearing of young, and increased reserves of vitamin A in the young, as judged by their survival times when transferred to a vitamin A-free diet. Fourth generation rats were successfully reared on diets containing 2, 4, or 8 per cent. butterfat, at which time the experiment was stopped. Only a small proportion of the fourth generation on the 1 per cent. butterfat diet was reared. Batchelder's results are in agreement with those obtained earlier by Sure (1930). That the difference was due to difference in the vitamin A content of the diet is indicated by the work of Deuel *et al.* (1945*b*) who carried out a similar experiment with margarine fortified with vitamin A replacing the butterfat of the Sherman B diet. They obtained good reproductive performance over 10 generations bred through the first litters and 8 generations through the second litters.

The above observation of Sherman *et al.* (1945) that the reproductive span of female rats was longer on a diet containing 9 I.U. vitamin A than at lower levels of vitamin A, translated into terms of carotene on the basis of 1 I.U. = 0·6 μg. carotene, gives a requirement of 5 p.p.m. of carotene which is in agreement with the findings of Fraps (1947).

It is now well established that utilisation of carotene and vitamin A by rats is influenced by α-tocopherol. References to this literature are to be found in a recent study by Lemley *et al.* (1947). There is some evidence also that soya bean lecithin contains a factor other than α-tocopherol which influences utilisation of carotene (Slanetz and Scharf, 1943; 1945; Scharf and Slanetz, 1944).

Foetal Supply

Rats

Dann (1932; 1934*a*) found values for the vitamin A stored in the foetal liver ranging from 0 when the mothers had been reared on a diet very low in carotene, to about 30 blue units when the mother had very large reserves of vitamin A. At weaning the liver reserves of vitamin A in young rats suckled by does on diets containing varying amounts of vitamin A (none deficient) were roughly proportional to the liver reserves of the doe, but since the amount of vitamin A stored by the young is very small compared with the doe's reserve it is considered that to a doe on a diet of natural foods the rearing of a litter is unlikely to cause a serious drain on her vitamin A reserves (Dann, 1934*b*).

Excess of Vitamin A

Rats

Sherwood *et al.* (1936; 1937) showed that daily administration of large doses of carotene, equivalent to 1500 and 3750 I.U. vitamin A, produced an upset in the oestrous cycle of female rats.

In 1927 Hartwell reported that female rats died of uterine haemorrhage when allowed to become pregnant on diets containing 12 per cent. of cod liver oil. Recently Moore and Wang (1945) confirmed the occurrence of uterine haemorrhage and failure of implantation or resorption in pregnant rats receiving a diet containing 20 per cent. of cod liver oil which supplied 1000 I.U. vitamin A daily, and showed that the results were similar when 30,000 to 45,000 I.U. vitamin A as vitamin A acetate or halibut liver oil in arachis oil were given daily within 10 days after coitus.

VITAMIN E

Restricted Supply

The nature of vitamin E, the effects of lack of vitamin E and theories of its physiological function have recently been the subject of an excellent review by Mason (1944).

Rats

Male rats deprived of vitamin E in early life show an irreversible degeneration of the seminiferous epithelium at the onset of sexual maturity, with resultant sterility. Vitamin E must be given before the 40th to 50th day of life if these changes are to be prevented. The need of the female rat for vitamin E does not arise until after conception and implantation. Females deprived of vitamin E from early life show normal oestrus, ovulation, conception and implantation of fertilised ova, but the foetuses die *in utero* and are resorbed. Repeated resorptions do not affect sexual functions or the ability to complete pregnancy when sufficient vitamin E is supplied, but prolonged depletion is associated with an increased requirement of vitamin E for the completion of gestation. If female rats deprived of vitamin E are given just sufficient at the beginning of gestation to enable them to produce live litters, the young are reared but tend to become paralysed towards the end of the suckling period. The occurrence of this muscular dystrophy can be prevented by administration of vitamin E to the mother or directly to the nursing young.

Mice

Resorption gestations occur also in mice deprived of vitamin E, but diets which cause resorption gestations in female rats and mice and testicular degeneration and sterility in male rats do not produce testicular degeneration or reduced fertility in male mice. Muscular dystrophy is seldom seen in suckling mice, but muscle lesions have been observed at autopsy, especially in mice 16 days of age or over.

Rabbits

In contrast to the early development of testicular degeneration in rats deprived of vitamin E, rabbits can show severe muscular dystrophy in the absence of testicular damage.

As many as 6 successive attacks of muscular dystrophy due to vitamin E deficiency have been produced and cured in rabbits without testicular degeneration.

Resorption of embryos and muscular dystrophy have been produced in guineapigs on diets deficient in vitamin E. Guinea-pigs

Muscular dystrophy has been observed also in puppies reared by bitches maintained on a diet low in vitamin E. Dogs

According to a recent investigation of the nutritional requirements of the Syrian hamster, young hamsters deprived of vitamin E collapse and die in from 4 to 18 weeks. Injection of α-tocopherol into collapsed animals is followed by recovery. Syrian hamsters

The foregoing statement is based upon the experimental work reviewed by Mason (1944), the investigations of Pappenheimer and Schogoleff (1944) and Kaunitz *et al.* (1944) on vitamin E deficiency in male rats, and Hamilton and Hogan's (1944) study of the hamster.

Destruction of Vitamin E

It is generally accepted that autoxidative rancidity in fats causes the destruction of vitamin E in the diet or in the digestive tract or both. Evidence has been presented by Kudryashov (1938) that administration of decomposition products of fat to pregnant rats on an adequate diet causes a greater disturbance of pregnancy than results from deprivation of vitamin E. But this has not been confirmed by other workers. Heptaldehyde administration causes foetal resorption in mice but not in rats.

The ability of plant foods, such as cereal germs, to prevent the symptoms of vitamin E deficiency in animals is due to their content of α-, β-, and γ-tocopherols. Of these the α-form is biologically the most active, and synthetic racemic α-tocopherol acetate has been adopted as the International Standard for vitamin E. Racemic α-tocopherol acetate and synthetic α-tocopherol have recently been used in investigations of the minimum daily requirement of laboratory animals for vitamin E. These are discussed below. For an account of investigations in which wheat germ oil was used as source of vitamin E, and of investigations in which preventive or curative doses of wheat germ oil or synthetic vitamin E have been administered, reference should be made to the review by Mason (1944).

Comparison of Different Levels of Tocopherol in the Diet

TESTICULAR DEGENERATION

Mason (1940) weaned young rats, whose mothers had received a vitamin E deficient diet during the last week of lactation, to the same vitamin E deficient diet consisting of casein 20, maize starch 48, lard 18, salts 4, yeast 8 and cod liver oil 2 per cent. In these "standardised" rats testicular degeneration occurs on an average 40 days after weaning, and resorption usually occurs in females experiencing their first pregnancy after a similar experimental period. Daily administration of synthetic α-tocopherol from the 10th day after weaning postponed the onset of testicular degeneration for 11 days when the dose was 0·0375 mg. and afforded complete protection when the dose was 0·07 mg. daily over a period of 70 days. There was still no evidence of degeneration 20 days after administration of tocopherol ceased. On the basis of mean bodyweight during the period over which the tocopherol was administered the fully protective dose of 0·07 mg. represents 0·33 mg. per kg. per day. Rats

Evans and his associates studied the requirements of the rat for α-tocopherol, using a standard vitamin E-low diet of casein 27, maize starch 35, lard 22, yeast 10, salts 4 and cod

liver oil 2 per cent. The diet, excluding the cod liver oil component, was allowed to stand for 2 weeks to permit the rancid substances of the lard to destroy traces of vitamin E. Evans *et al.* (1939) gave 2 groups of 6 male rats a vitamin E-low diet from weaning. One group received a daily supplement of 0·75 mg. α-tocopherol. After 4 months each male was offered an oestrous female. Twelve matings of each animal in each group resulted in the production of 521 living young of normal weight in the tocopherol group, but none in the unsupplemented group. When the male rats were killed at 277 days of age testicular degeneration was found in the unsupplemented group but not in the group receiving α-tocopherol.

Evans and Emerson (1943) weaned young rats from stock mothers to their standard diet low in vitamin E. One group received no supplement. Others were given 0·10, 0·25 and 0·75 mg. synthetic α-tocopherol acetate six times weekly. A control group was weaned

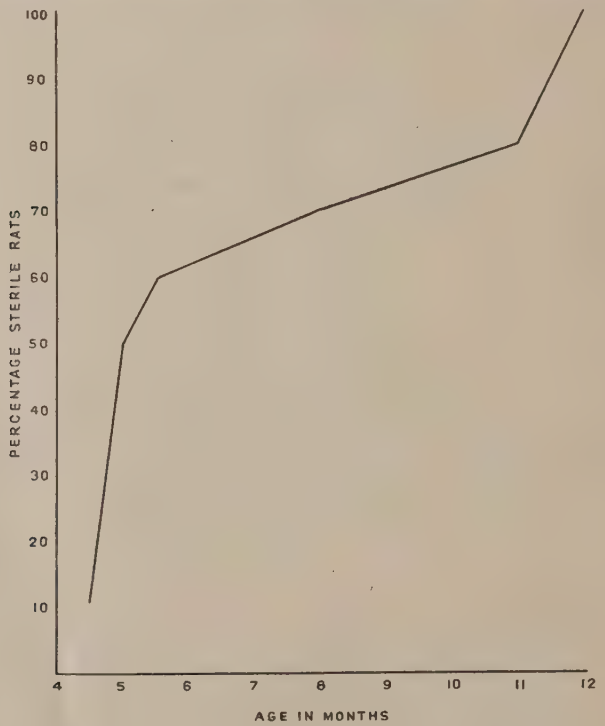

FIG. 6.—Male Sterility on Diet Supplemented with 0·1 mg. α-Tocopherol Acetate.

to the stock diet. Males were offered oestrous females at fortnightly to monthly intervals from the age of 4 months until they were 16 months old. All males on the basal vitamin E-deficient diet were sterile after the first mating. Of those receiving 0·10 mg. α-tocopherol acetate, half were sterile after the second mating and matings were uniformly sterile in the last 4 months. The performance of males receiving the 0·25 mg. dose equalled that of males on the 0·75 mg. dose during the first nine months of life, but from the 10th to 16th month the latter group sired approximately $3\frac{1}{2}$ times the number of litters sired on the 0·25 mg. dose. The fertility of the male rats on the vitamin E-deficient diet supplemented with 0·75 mg. α-tocopherol acetate surpassed that of the control rats on stock diet. It was concluded that

the requirement of the male rat for reproductive efficiency lay between 0·25 and 0·75 mg. α-tocopherol acetate daily.

The ages at which the 10 male rats used by Evans and Emerson became sterile while receiving a daily supplement of 0·1 mg. α-tocopherol acetate are shown in Figure 6.

The fact that Mason's rats killed at about 3½ to 4 months of age showed no testicular degeneration when their diet was supplemented with 0·07 mg. α-tocopherol daily, does not necessarily preclude the possibility of sterility occurring at a later date as found by Evans and Emerson (1943).

Escudero and Herraiz (1940-41) gave male rats, whose mothers had received a diet low in vitamin E during the last week of lactation, a low-fat diet deficient in vitamin E from weaning, supplemented with amounts of α-tocopherol acetate ranging from 0·006 mg. to 0·6 mg. daily. After 22 weeks on the experiment, fertility was tested by matings with normal females of proved fertility. About a week later the animals were killed and their testes examined histologically. The percentages of fertile matings are shown in Table XVII.

TABLE XVII

Effect of Supplements of α-Tocopherol on Fertility of Male Rats

Supplement of α-tocopherol acetate, mg.	0·006	0·01	0·03	0·06	0·3	0·6
Fertile matings, per cent. . . .	0	25	100	75	62	66

Testicular degeneration occurred in the unsupplemented control group and in the groups receiving 0·006 and 0·01 mg. α-tocopherol acetate. Slight testicular degeneration was noted also in the groups receiving supplements of 0·03 and 0·6 mg. The presence of lesions in the latter group is attributed to an excess of vitamin E, but unfortunately the testes in the two intermediary groups were not examined histologically. The significance of these findings is not clear. Under the conditions of this experiment a supplement of 0·03 mg. α-tocopherol acetate was sufficient to preserve fertility to an age of about 6½ months in male rats on a diet low in vitamin E. Since testicular degeneration was observed it may be presumed that fertility might not have been maintained at subsequent matings.

Engel and Bretschneider (1943) investigated the effect on male fertility of supplementing with tocopherol a low-fat, vitamin E-deficient diet and Evans and Emerson's vitamin E-deficient diet. Their results were inconclusive with the former. They were negative with the latter: the rats remained fertile for 7 months without a supplement, presumably because they were 3 months old before being given the experimental diets.

PREGNANCY

Rats

In the experiment by Mason (1940) reported above female rats were given synthetic α-tocopherol from the 10th day after weaning until the 10th day after the first gestation. At the lower level of intake, 0·0375 mg. daily, only 2 out of 5 rats showed live foetuses *in utero* at the 16th day of gestation. At the higher level, 0·07 mg., all the rats delivered litters of 7 to 10 pups at term. It is concluded that the minimum protective dose lies between these two levels of intake for both male and female rats. On the basis of mean bodyweight during the period over which the tocopherol was administered the fully protective dose of 0·07 mg. represents 0·48 mg. per kg. per day for the females.

In the investigation of Evans and Emerson (1943) described above female rats in the various groups were bred with normal males at the first pre-oestrus after 90 days on the

experimental diets. Breeding was continued until 3 successive litters were secured. All the untreated females underwent typical resorption gestations. At all three levels of α-tocopherol supplementation, 0·10, 0·25 and 0·75 mg. six times weekly, all animals showing the implantation sign littered and produced litters of about the same size and same average birthweight as control rats on the stock diet. Only those rats receiving the 0·75 mg. dose were protected against the typical dark-brown discoloration of the uterus caused by vitamin E deficiency. This level was required also for the prevention of paralysis in the young, but even this amount did not give complete protection in the 3rd litters. The results are presented in Table XVIII.

Evans and Emerson's estimates of the requirements of rats are many times higher than those obtained by Mason (1940), but they point out that Mason's experiments were of short duration, his animals were killed at the age at which the breeding tests of Evans and Emerson were initiated, he did not study the tocopherol requirements during lactation for the prevention of muscular dystrophy in the suckling young, and, further, the basal diets used in the two investigations were different.

If the criterion of adequacy for female rats had been restricted by Evans and Emerson to the amount of α-tocopherol necessary for the production of live litters, as was done in Mason's experiment, then the supplement of 0·1 mg. α-tocopherol acetate six times weekly would have been considered adequate. This corresponds to an intake of 0·6 mg. α-tocopherol per kg. bodyweight according to the calculations of Mason (1944), and is at least of the same order as his estimated requirement of 0·48 mg. per kg. When uterine pigmentation is taken as criterion the position is still not clear. Moore (1940) found that administration of a weekly dose of 1 mg. α-tocopherol to 8-week-old rats which had been on a vitamin E-deficient diet from weaning was sufficient to prevent uterine pigmentation over an experimental period of 29 weeks, but 0·3 mg. weekly was insufficient. The vitamin A reserves in the livers of rats receiving the lower dose were only about half that of those getting the 1 mg. supplement. According to Mason's calculations (1944) these levels correspond to a minimum necessary intake of between 0·3 and 1·0 mg. α-tocopherol per kg. bodyweight, which is considerably below the level which Evans and Emerson reported as necessary to prevent the occurrence of uterine pigmentation in rats which had borne 3 litters. The requirements of vitamin E to prevent uterine pigmentation in the female rat may be increased by the strain of bearing young.

Mason (1944) has suggested that the induction of rancidity with the intention of destroying traces of vitamin E in experimental diets, adopted by Evans and Emerson, may inadvertently decrease the amount of dietary tocopherol available for preventing the pathognomonic signs of vitamin E deficiency and give higher values than normal for minimal requirements.

There is evidence that the requirement of the rat for vitamin E is much less on a low-fat diet than on a diet containing appreciable amounts of fat (Gottlieb *et al.*, 1943; Homrich, 1943; Emerson and Evans, 1944), but this could scarcely account for the different results obtained by Mason (1940) and Evans and Emerson (1943) with diets containing 18 and 22 per cent. of lard, respectively.

Recent investigations of Scharf and Slanetz (1944) indicate that soya bean lecithin contains a substance which influences the utilisation of α-tocopherol by female rats.

Dogs

Elvehjem *et al.* (1944) found that a diet of evaporated milk supplemented with Fe, Cu, Mn and cod liver oil allowed normal growth in dogs but was inadequate for reproduction in females. The pups died within a few days of birth, showing cranial haemorrhages. A weekly supplement of 10 mg. α-tocopherol acetate or 25 mg. α-tocopherol during gestation appeared to reduce the incidence of haemorrhage in the young, but they did not survive more than a

TABLE XVIII

THE EFFECT OF SUPPLEMENTS OF α-TOCOPHEROL ACETATE ON REPRODUCTION AND REARING OF YOUNG RATS

Diet	No. Rats	No. Litters	Total No. Living Young	Litter Size	Av. Wt. at Birth, g.	Percentage of Young Surviving on 21st Day*	Av. Wt. of Young at 21 Days, g.	Percentage of Young Surviving on 30th Day	Av. Wt. of Young at 30 Days, g.	Percentage of Young Paralysed	Percentage of Paralysed Young Recovering by 30th Day
1st Litter											
Vitamin E-deficient	15	0	—	—	—	—	—	—	—	—	—
+0·1 mg. α-tocopherol acetate	15	15	133	8·9	5·7	75	46	43	62	100	23
+0·25 mg. „ „ „	15	15	123	8·2	5·8	70	51	67	85	24	82
+0·75 mg. „ „ „	15	14	115	8·2	5·8	84	53	84	92	0	—
Stock	14	14	130	9·3	5·9	89	47	89	83	0	—
2nd Litter											
Vitamin E-deficient	13	0	—	—	—	—	—	—	—	—	—
+0·1 mg. α-tocopherol acetate	14	12	110	9·2	5·9	73	46	48	53	100	10
+0·25 mg. „ „ „	14	14	122	8·7	5·8	82	54	80	85	54	58
+0·75 mg. „ „ „	14	13	114	8·8	6·2	93	54	92	88	0	—
Stock	14	11	87	7·9	6·3	89	48	93	88	0	—
3rd Litter											
Vitamin E deficient	8	0	—	—	—	—	—	—	—	—	—
+0·1 mg. α-tocopherol acetate	12	9	50	5·6	5·9	39	43	3	45	100	0
+0·25 mg. „ „ „	14	10	64	6·4	5·4	75	47	48	55	100	0
+0·75 mg. „ „ „	14	10	82	8·2	5·6	82	52	80	88	30	100
Stock	14	8	58	7·2	5·9	75	56	73	88	0	—

*Litters were reduced to 6.

few days. A weekly supplement of 40 mg. α-tocopherol resulted in the production of a litter of 4 normal pups, all of which were reared. Muscular dystrophy was observed in one pup at 4 weeks of age. This level of α-tocopherol is stated to be equivalent to an intake of approximately 1 mg. per kg. bodyweight daily for the dogs used.

Guinea-pigs

Pappenheimer and Goettsch (1941) reported that guineapigs on a diet containing insufficient vitamin E to protect against muscular dystrophy were unable to complete successful pregnancies even when given a weekly supplement of 5 or 10 mg. α-tocopherol acetate, although these supplements prevented the occurrence of muscular dystrophy. Resorption of the embryos occurred in a high proportion of cases, accompanied by haemorrhagic necrosis of the placenta. The basal diet consisted of casein 20, sucrose 15, maize starch 35, yeast 5, salt mixture 4, lard 8, cod liver oil 2 and cellulose 20 parts, supplemented with 5 ml. tomato juice and 25 g. fresh lettuce daily. Further experiments are needed to establish the minimum dose of α-tocopherol required for successful completion of pregnancy.

Foetal Supply: Mammary Transfer

Rats

Mason (1940) has shown that in young male rats borne by mothers receiving large supplements of vitamin E and suckled by stock rats the onset of testicular degeneration when the young were weaned to a vitamin E-deficient diet was not delayed. In young borne by stock mothers and suckled by females receiving high levels of vitamin E, the onset of testicular degeneration after weaning on to a diet low in vitamin E was delayed by about 47 days. Similar experiments with female young bred 40 days after weaning showed that neither mammary transfer nor placental transfer of vitamin E from animals receiving large amounts of vitamin E during lactation or pregnancy prevented resorption of foetuses during the first pregnancy, if the young rats were weaned on to a diet low in vitamin E.

Further long-term experiments are clearly needed to determine the requirements of laboratory animals for vitamin E, taking into account all the other components of the diet known to influence these requirements.

VITAMIN K AND "GRASS JUICE FACTORS"

Rats

Synthesis of vitamin K in the intestinal tract of the rat has been demonstrated by several investigators and many consider inclusion of this vitamin in purified diets unnecessary. Recently Brown *et al.* (1947) reported the occurrence of brain haemorrhages in 52·2 per cent. of the offspring of female rats fed on a purified diet of casein 25, cerelose 67, wood pulp 3 and salt mixture 5 per cent., supplemented with vitamins A, D and E and all the known essential members of the B complex except folic acid. Brain haemorrhage did not occur in the young when 10 per cent. lard replaced cerelose or when 2·5 mg. of 2-methyl-1 : 4 naphthaquinone was included per 100 g. of diet or when both these additions were made to the diet of the does. The percentage of young reared to weaning was increased by these supplements. The coagulation time of the blood of 12 mothers which produced young showing haemorrhages and of 6 of these young was determined and found to be within the normal range. Chick assays indicated the presence of only a very small amount of vitamin K in the lard. Brown *et al.* concluded that the brain haemorrhages were not entirely due to deficiency of vitamin K and suggested that under normal conditions some substance which prevents capillary fragility is not synthesised in the body when the diet is low in both fat and vitamin K. In a second test brain haemorrhages developed in only 10 out of 72 young which were born to 8 females and Brown *et al.* were unable to account for the low incidence of haemorrhages in this series.

Brown *et al.* compared their findings with those of Moore *et al.* (1927) who observed brain haemorrhages in the young of rats reared on a diet deficient in B vitamins (containing 3 per cent. of crisco and 2 per cent. of cod liver oil) and those of Jervis (1942) who found brain haemorrhages in the young of female rats reared on a diet high in cystine and low in choline (containing 10 per cent. crisco) (see page 72). A common etiology is suggested.

Rabbits Guinea-pigs

Moore *et al.* (1942) found that a diet containing alfalfa, which permitted the production of normal rabbit litters, caused abortion when acetone-extracted alfalfa was used. This was shown to be due to removal of vitamin K. Rabbits which had undergone a successful pregnancy on a vitamin K-deficient basal diet supplemented by 1 mg. of vitamin K on alternate days aborted in two successive pregnancies when the supplement was withheld, and later had a successful pregnancy with production of a live litter when vitamin K was again included in the diet. Placentas were recovered from 5 of the aborted foetuses. These showed haemorrhage in the decidual plates. The basal diet used in this experiment consisted of acetone-extracted casein 150, wheat starch 375, cottonseed oil 112·5, salts 32·5, acetone-extracted yeast 32·5, agar 32·5, cod liver oil 15, acetone-extracted alfalfa powder 750 parts, with 1 drop of wheat germ oil and 5 mg. ascorbic acid daily.

Hogan and Johnson (1936) found that a ration of ground oats 60, skimmed milk powder 33, wheat germ oil 4, cod liver oil 1, NaCl 1 and $CaCO_3$ 1 per cent. supported growth but was inadequate for reproduction in rabbits and guineapigs. Guineapigs had irregular oestrous cycles, few became pregnant and some aborted. Rabbits produced young, few of which survived. Extensive haemorrhages occurred in the young rabbits. Addition of the juice or alcohol- and ether-extracts of the juice of young cereal grasses to the diet permitted successful reproduction. A combination of the two extracts was more effective than either alone. Later Hogan and Hamilton (1942) found that addition of vitamin K to a purified ration for rabbits and guineapigs permitted the rearing of litters. Chamorro (1946) reported that administration of vitamin K, or vitamin C, to immature female rabbits provoked enlargement of the uterus with congestion, and of the mammary gland with secretion of a clear fluid.

Nordfeldt (1942) recently presented evidence that fresh grass contains a factor or factors essential for fertility in the rabbit and viability of the young which is not identical with vitamin A, D or E nor supplied by dried yeast. Bradbury (1944) investigated the "rabbit ovulating factor" present in plant juice. The nature and relationship of these "factors" is not known.

VITAMIN C

Guinea-pigs

That the guineapig needs vitamin C in the diet if scurvy is to be prevented is well established and the beneficial effect of fresh green food in the diet has long been recognised. Recent investigations of the requirements of the guineapig indicate, however, that green food contributes some factor or factors other than vitamin C essential for normal growth.

Restricted Supply

SPERMATOGENESIS

Guinea-pigs

The presence of vitamin C has been demonstrated in the male genital organs of normal guineapigs but not in those of guineapigs suffering from scurvy (Giroud and Leblond, 1934). Zimmett (1939) showed that in male guineapigs on a diet deficient in vitamin C the indophenol-reducing capacity of the ejaculate varied inversely with the degree of deficiency. In animals on a deficient diet the coagulation time of the ejaculate was increased and the number, motility and longevity of the spermatozoa were decreased. Marked changes in the cholesterol content of the testicles and ejaculate occurred during the onset of scurvy.

PREGNANCY

Guinea-pigs

Some workers have found that pregnancy delays the onset of scurvy in guineapigs on a scorbutic diet (Mouriquand and Schoen, 1933; Fujino, 1933; Bourne, 1935). Mouriquand and Schoen's results suggested that the foetus could synthesise vitamin C and so protect the mother from scurvy. Bourne (1935) found that virgin female guineapigs injected with the luteinising hormone of the anterior pituitary were also protected from the effects of vitamin C deficiency, and suggested that the *corpus luteum* of the guineapig as well as the foetus could synthesise vitamin C. Laporta and Rinaldi (1935), however, reported that controlled experiments with guineapigs gave no evidence of synthesis of vitamin C by the *corpus luteum* or the foetus, and Mouriquand *et al.* (1936), finding no ascorbic acid in the foetuses of guineapigs on a scorbutic diet and signs of scurvy in the foetuses, concluded that the foetus could not synthesise vitamin C. Saffry and Finerty (1939) stated that pregnant guineapigs maintained on a diet low in vitamin C aborted. Injection of *corpus luteum* extract daily did not prolong the period of gestation, nor did such injections induce pseudopregnancy in non-pregnant guineapigs. The abortions, however, may have been caused by some deficiency other than of vitamin C. Guha and Gosh (1935) reported that embryonic guineapig tissue in the early stage of development was capable of synthesising vitamin C from mannose *in vitro*, but Euler *et al.* (1935) were unable to confirm this finding.

The ripe *corpus luteum* of guineapigs (Huszák, 1933), and foetuses and suckling young of guineapigs (Giroud *et al.*, 1936; Brieger, 1942) are normally rich in vitamin C. Brieger emphasised the importance of an ample supply of vitamin C in the mother's diet during pregnancy and lactation.

Crampton (1947) pointed out that several diets which have been used for the bioassay of vitamin C have been found, when fortified with ascorbic acid, to be inadequate for pregnancy or normal growth of young guineapigs to maturity. Changes in the ovaries and the occurrence of abortions or resorptions of embryos in guineapigs on vitamin C-free diets have been reported by several workers (Mouriquand and Schoen, 1933; Fujino, 1933; Kramer *et al.*, 1933; Mouriquand, 1935; Giedosz and Rychlik, 1936; Saffry and Finerty, 1939). But these cannot be taken as pathognomonic of vitamin C deficiency in the absence of evidence to show that their occurrence is prevented by inclusion in the diet of pure vitamin C.

Comparison of Different Levels in the Diet

PREGNANCY

Guinea-pigs

Kramer *et al.* (1933) observed that the degree of degeneration of the ovaries was less when guineapigs were given 5 ml. of orange juice or tomato juice daily than when they received less than this amount and that pregnancies occurred only in animals receiving 3 or 5 ml. of orange juice or tomato juice, but abortions and resorptions occurred in all groups irrespective of the supplement and in no group was a live litter born. The basal vitamin C-free diet used in this investigation consisted of a mixture of equal parts of rolled oats and wheat bran 59, heated skimmed milk powder 30, butterfat 8, cod liver oil 2 and NaCl 1 per cent. Tests with rats indicated that it was adequate for normal growth and reproduction.

Crampton and Bell (1947) compared the effects of 5 mg. of pure ascorbic acid and fruit juices in amounts calculated to supply 5 mg. vitamin C as daily supplements to a basal diet of oats 15, wheat 13, beet pulp 25, oil meal 12·5, skimmed milk 15, fishmeal 5, brewer's dried yeast 10, bone char 4 and iodised salt 0·5 per cent. Vitamins A, D and E were given weekly in maize oil. At the same time the effects of adding hay or fresh grass were compared in groups arranged on the latin square plan. The diets were given to groups of 5 mature female

guineapigs which had previously had 2 or 3 litters in the breeding colony. When the females were grouped according to the supplement of roughage, irrespective of the type of vitamin C supplement, the group given fresh grass was the only one in which neither haemorrhage nor abortion occurred. When grouped according to the supplement of vitamin C, irrespective of the roughage supplement, it was found that the largest number of abortions occurred among females given pure ascorbic acid alone or in synthetic orange juice; there was none in the group given natural orange juice. The largest number of haemorrhages occurred in the group given lemon juice.

The trial was repeated but mature females were not available for the replicate and primigravid females were used. In this series the supplements of fat-soluble vitamins, which were given at levels of 425 I.U. vitamin A, 48 I.U. vitamin D_2 and 3 mg. α-tocopherol daily in the first test, were doubled. Abortion did not occur in any group and the only instances of haemorrhage were among those given pure ascorbic acid alone.

Assays made during the experiment showed that the actual amount of vitamin C supplied by the lemon juice was less than the 5 mg. daily which had been anticipated. Since pregnancy occurred at this level and did not occur in the experiment of Kramer *et al.* (1933) when the amount of orange juice probably supplied about 2 mg. vitamin C daily, Crampton and Bell concluded that female guineapigs on their basal diet require somewhere between 2 and 5 mg. daily for pregnancy. They emphasised, however, that vitamin C *per se* was obviously not the limiting factor in their reproduction tests. The superior performance of the guineapigs in the second replicate was not attributed to the increased amounts of fat-soluble vitamins given. Crampton and Bell believed the difference to be due to depletion of reserves of the older guineapigs caused by the strain of previous litters.

LACTATION

Rasmussen *et al.* (1938) showed with guineapigs that on a diet limited in vitamin C content a daily dose of 40 mg. ascorbic acid from 17 days before parturition raised the ascorbic acid content of the milk from 110 mg. per quart to 711 mg. per quart. Guinea-pigs

VITAMIN B COMPLEX COMPONENTS AND CHOLINE

In this review of the available evidence on the requirements of laboratory animals for individual components of the B complex during the reproductive period no useful purpose would be gained by studying earlier investigations which were hampered by lack of these vitamins in pure form. References to older work (with the exception of the paper by Drummond *et al.*, 1938) are to be found in the review by Garry and Stiven (1936).

Restricted Supply of Vitamin B_1

OESTROUS CYCLE

Coward *et al.* (1942) showed that a daily supplement of 6 µg. vitamin B_1 was insufficient to induce normal oestrous cycles in female rats maintained on a diet deficient in vitamin B_1 until cornified cells were absent for 10 days. A dose of 12 µg. daily was the lowest amount that would restore the rhythm of the oestrous cycle. The requirement for satisfactory reproduction and rearing of the young was not ascertained because the diet, even when supplemented with large doses of vitamin B_1, was inadequate for the production and rearing of young, presumably because of some other deficiency. According to Drill and Burrill (1944), the effect of deprivation of vitamin B_1 on ovarian function is due to the inanition produced by loss of appetite. Rats

TRANSMISSION FROM MOTHER TO FOETUS

Rats Westenbrink and Veldman (1941) studied the relation between the vitamin B_1 pyrophosphate content of the mother and foetus or newborn young. This paper was not available for consultation but from a published abstract it would appear that when female rats were deprived of vitamin B_1 during pregnancy the embryo was supplied with vitamin B_1 at the expense of the mother.

There is some evidence that a high riboflavin content in the maternal diet increases the ability of the young to withstand deprivation of vitamin B_1 in later life (Ellis and Zmachinsky, 1937; Ellis *et al.*, 1943).

Level of Vitamin B_1 in Stock Diet

Rats Williams and Spies (1938) calculated that Sherman's B diet, which permits excellent reproduction in rats, contained 4 μg. vitamin B_1 per g.

Excess of Vitamin B_1

Rats In 1937 Perla first presented evidence that excessive supplementation of a good diet of natural foods with vitamin B_1 had an adverse effect on the ability of lactating rats to rear their young. Williams and Spies (1938) criticised Perla's results and stated that successful reproduction had been obtained over 3 generations with rats given a stock diet supplemented with 80 to 1000 μg. vitamin B_1 daily. Sure (1939) also described an adverse effect on the rearing of young by does receiving a diet already rich in vitamin B_1 supplemented with 400 to 800 μg. vitamin B_1 daily, but the effect was not apparent until the third generation, when only a small percentage of young were reared.

Perla has since presented evidence to show that the toxic effect on rats of 400 μg. vitamin B_1 daily can be prevented by simultaneous administration of 2 mg. Mn as $MnCl_2$ and that in the absence of a high intake of vitamin B_1 this amount of Mn adversely affected the rearing of litters (Perla and Sandberg, 1938-39; Perla, 1939).

Mice Cerecedo and Vinson (1944c) were unable to reproduce Perla's results with mice. Three generations of mice were successfully reared on a diet of purina dog chow supplemented with vitamin B_1 and/or $MnCl_2$, although the daily intake of vitamin B_1 during lactation was approximately 2000 μg. in the groups receiving the vitamin B_1 supplement and the intake of Mn was about 8 mg. daily in the groups receiving the supplement of $MnCl_2$. The only adverse effect noted was in the second generation of the vitamin B_1-supplemented group, when all the mothers destroyed their first litters. They successfully weaned their second litters. Ehrismann (1939) reported that feeding MnO_2 to mice decreased litter size and increased the number of stillborn young and pre-natal mortality, but had no effect on the rearing of the young.

Rats Evidence that under certain conditions addition of vitamin B_1 to the diet of does may have an adverse effect on the health of suckling young was recently provided by the work of Richards (1945). In these experiments the basal diet used consisted of white flour 102, commercial casein 40, dried brewer's yeast 3·2, salt mixture 3·3 and margarine 18 parts, with addition of vitamins A and D as radiostoleum, E as α-tocopherol, KI, $MnSO_4$ and a small amount of synthetic vitamin B_1. This diet provided 0·33 g. Ca and approximately 594 μg. vitamin B_1 per 1000 Cal. A control group was reared on the basal diet and other groups were given chalk to increase the Ca content to 0·81 g. per 1000 Cal. and/or a supplement of vitamin B_1 to raise the intake to 11,279 μg. per 1000 Cal. An intermediate group was given the Ca supplement and enough vitamin B_1 to raise the intake to 1664 μg. per 1000 Cal. All

the does were given 10 or 20 ml. of milk daily during lactation, and litters were reduced to 8 at birth. In the groups receiving the medium or high intakes of vitamin B_1 plus chalk, mortality was high in the litters of does getting 10 ml. of milk daily; where the doe received 20 ml. of milk there were fewer deaths among the young but a convulsive syndrome was observed usually from about the 19th day onwards. A few cases were observed also in the group on the high intake of vitamin B_1 without added chalk. Because of the resemblance to the symptoms of vitamin B_6 deficiency in nursing young, further breeding tests were made with the rats from the 3 supplemented groups, which showed that the occurrence of fits in the young could be prevented by daily administration of 40 μg. vitamin B_6 to the doe from parturition.

Restricted Supply of Riboflavin

OESTROUS CYCLE

Coward *et al.* (1942) during biological assays took rats which had been maintained from a weight of about 60 g. on a diet containing little or no riboflavin and which had ceased to grow after 28 to 52 days' depletion, and gave them daily doses of riboflavin ranging from 1 to 7·5 μg. for a period of 21 days. Rats receiving 7·5 μg. riboflavin or less and 2 rats which were not given any supplement were found to be in anoestrus. Out of 3 rats given 30 μg. riboflavin 2 showed cycles regularly and the third was in anoestrus. After a period of anoestrus ranging from 23 to 32 days, oestrus was restored in all the anoestrous rats by daily administration of 20 μg. riboflavin or 10 ml. milk. Rats

ANATOMICAL CHANGES

Shaw and Phillips (1941) described the occurrence of atrophy of the testes in rats given a diet low in riboflavin and Colonge and Raffy (1947) marked abnormalities in the reproductive organs of a male and female rat completely deprived of riboflavin. Rats

PREGNANCY

In the experiment reported by Coward *et al.* (1942) female rats which had previously been severely depleted of riboflavin were treated with riboflavin or milk to restore the normal oestrous cycle and mated. Although they were then given a complete diet or a supplement of milk they either failed to produce litters or failed to rear them, indicating the irreversibility of the changes produced by continued depletion during the first quarter of the rat's reproductive life. Rats

In a series of papers Warkany and his co-workers (Warkany and Nelson, 1940; 1941; 1942*a*, *b*; Warkany *et al.*, 1942; Warkany *et al.*, 1943) and Noback and Kupperman (1944) reported the occurrence of congenital malformations in the young of female rats maintained on a rachitogenic diet of yellow maizemeal 76, wheat gluten 20, $CaCO_3$ 3, NaCl 1 per cent., supplemented with 60 I.U. of vitamin D as viosterol every 10th day to prevent the occurrence of rickets. The malformations, shortening of the mandible, cleft palate, defective growth of the long bones and fusion of ribs, fingers and toes, could be prevented by inclusion of 2 per cent. of dried pig liver in the diet. Warkany and Schraffenberger (1943) subsequently succeeded in reproducing the condition in females on a diet deficient in riboflavin. The diet consisted of sucrose 68, purified casein 18, vegetable oil 10 and salt mixture 4 per cent., supplemented by vitamin A, 0·8 mg. vitamin B_1 and vitamin B_6, 1 mg. Ca pantothenate, 10 mg. nicotinamide and 100 mg. choline chloride. Rats given this diet at 3 months of age maintained their weight or gained from 4 to 15 g. during the first 2 weeks. They also showed regular oestrous cycles for several weeks. When mated to normal stock males 19 out of 21

became pregnant within 3 weeks. Of these pregnancies 11 ended in resorption and 8 litters were delivered at term. The pregnancies caused such weight losses in 11 animals that these were considered unfit for further breeding. Of the 8 remaining rats, 4 did not show oestrous cycles within 8 weeks after the first pregnancy, 4 underwent a second pregnancy and 3 produced litters. Of the total 74 young obtained, 3 first litter young and 7 second litter young showed abnormalities of the type previously described.

In a subsequent investigation (Warkany and Schraffenberger, 1944*a*) the diet was modified by inclusion of α-tocopherol and vitamin K. Female rats aged 3 months were given the deficient diet. After 3 weeks when they showed oestrus they were mated with stock males. Some became pregnant immediately, others only after several matings and some were sterile. From 54 females, 25 first and 1 second litter had been obtained by the time of publication. These consisted of 137 young, of which 33 were abnormal. From a control group of 34 females which received a supplement of 0·8 mg. riboflavin per 100 g. of diet, 27 first, 2 second and 1 third litter were obtained, containing a total of 120 normal young. In the deficient diet group about half of the females ceased to have oestrous cycles after several weeks on the diet. Small graded doses of riboflavin were given until oestrus was resumed and a bodyweight of about 200 g. achieved. Attempts in this way to " restore " females which had been on the riboflavin-deficient diet for more than 4 months failed. " Restored " rats were bred. In 7 a litter resulted ; in 4 riboflavin was discontinued after conception, but the 3 other rats were given small amounts during pregnancy. Of the 52 young obtained, 28 or 53·9 per cent. were abnormal. Warkany (1945) summing up the results of these experiments says that the malformations are not determined before the 13th day of pre-natal life. They occur before or at the stage of chondrification, which in the rat begins on the 14th or 15th day. A critical stage, therefore, appears to exist between the 13th and 15th day in which the presence or absence of sufficient riboflavin influences the development of the embryo.

Comparison of Different Levels of Riboflavin in the Diet

Records of Performance

Rats

Ellis *et al.* (1943) found no significant difference in the reproduction records of 2 generations of rats reared on diets differing only in their riboflavin contents, which were 3, 6·5 and 10 μg. per g. of diet, respectively, or 0·9, 2·0 and 3·0 μg. per food Calorie. Nor was the riboflavin content of the organs of adult rats increased by increasing the riboflavin content of the diet above 3 μg. per g. The young of females on the diet containing the highest level of riboflavin, however, grew better after weaning to the family diet than the young of the group receiving the lowest intake of riboflavin. The reproduction records are shown in Table XIX.

TABLE XIX

Average Reproduction Records of Female Rats with Different Supplements of Riboflavin

Riboflavin in Diet, μg. per g.	3	6·5	10
Age of female rats at birth of first young (days)	(45)* 117±2·5	(43) 133±5·2	(46) 120±2·6
Total number young born per female	(48) 29·0±1·6	(48) 21·5±1·1	(48) 24·3±1·5
Total number young reared per female	(48) 15·5±1·1	(48) 12·4±0·8	(48) 15·6±1·2
Reproductive life (days)	(48) 251±15	(48) 201±11	(48) 228±14
Average weight of young at 28 days, g.			
Male	(407) 43·9±0·08	(333) 42·6±0·1	(453) 43·7±0·08
Female	(389) 41·8±0·08	(328) 42·3±0·1	(456) 43·2±0·08

* Figures in brackets are numbers of cases.

Restricted Supply of Vitamin B_6

MALE STERILITY

Emerson and Evans (1940) reported that male rats reared on a diet deficient in vitamin B_6 were sterile. Rats

PREGNANCY AND LACTATION

The effect of deficiency of vitamin B_6 on the ability of does to rear their young has been described by Daniel *et al.* (1942). This group of workers had previously observed that a diet used in vitamin B_1 assays, when supplemented with vitamin B_1, permitted normal growth and reproduction in female rats, but a convulsive syndrome was observed in the suckling young (Kline *et al.*, 1938). Further investigation showed that administration of vitamin B_6 to the mother or to the young resulted in normal young. If the young from experimental females were suckled by stock females on a stock diet they were reared successfully, and, conversely, if stock young were given to the experimental females to suckle, convulsions occurred, but in some cases the onset of symptoms was delayed, and a small proportion of the animals survived. The condition was produced also when stock females were transferred a few days before parturition to diets deficient in vitamin B_6, consisting of purified casein 18, salts 4, cod liver oil 1, or irradiated ergosterol and a vitamin A concentrate, with or without maize oil 5 and liver extract 1 and made up to 100 parts with sucrose, supplemented with 200 μg. vitamin B_1, 300 μg. riboflavin and 500 μg. Ca pantothenate per 100 g. diet. Female rats reared to maturity on this diet supplemented with vitamin B_6 reared normal young if vitamin B_6 was continued during pregnancy and lactation (200 μg. per 100 g.), but if vitamin B_6 was omitted from before mating or before parturition the convulsive syndrome appeared and the young died after about the 13th day of lactation. There was no apparent injury to females maintained on diets devoid of vitamin B_6 during pregnancy and lactation. After the loss of 2 or more litters it was possible for such mothers to rear normal litters if the diet was supplemented with vitamin B_6. When pregnant females just before parturition were given the purified diet without liver extract, modified by addition of vitamin B_6 and omission of riboflavin, the young became denuded of hair and stunted, but survived for 5 or 6 weeks. When pantothenic acid was omitted, sudden respiratory failure with collapse and death occurred in the young between the 10th and 20th day. In neither case were the effects similar to those resulting from deficiency of vitamin B_6. Patton *et al.* (1944) also described the occurrence of spontaneous convulsions in young rats suckled by mothers maintained from parturition on purified diets devoid of vitamin B_6. When vitamin B_6 was included in the mother's diet at levels varying from 25 to 150 μg. per day no case of spontaneous seizure was observed in the young. Rats

Sure (1945) maintained groups of male and female rats from weaning on a purified diet of casein 22, butterfat 5, salt mixture 5, and various levels of rice by-products as source of vitamin B_6, made up to 100 with glucose. The diet was supplemented daily with 20 μg. each of vitamin B_1 and riboflavin, 6 mg. choline chloride and 200 μg. Ca pantothenate. From mating the quantities of vitamin B_1 and riboflavin were increased to 50 μg. daily. During lactation these were further increased to 120 μg., the choline to 9 mg. and the pantothenic acid to 600 μg. daily. Vitamins A, D and E were supplied also, and cystine was added to the diet from mating. When rice polishings were given at a level of 5 per cent. as source of vitamin B_6, 31·3 per cent. of young were reared, compared with 68·3 per cent. when 15 per cent. of rice polishings was given. The latter was estimated to provide 92·4 μg. vitamin B_6 daily on the basis of a food consumption of 20 g. during lactation.

Reference has been made already (page 66) to the work of Richards (1945) in which the occurrence of fits induced in the suckling young of female rats on a diet containing an excess of vitamin B_1 and high in Ca could be prevented by daily administration of vitamin B_6 to the doe from parturition.

Restricted Supply of Pantothenic Acid

PREGNANCY AND LACTATION

Rats

Jukes (1940) reported that when black female and male rats, reared on a purified diet from which pantothenic acid was excluded, were mated, one-third of the females resorbed their foetuses; the others produced litters but the offspring died within 4 days of birth without suckling having been observed. But the basal diet, which consisted of sucrose 60, acid-washed casein 25, crisco 8 and salt mixture 5 parts, supplemented by fish oil blend and shark liver oil as sources of vitamins A and D, maize oil, and vitamin B_1 0·2 mg., riboflavin 0·5 mg., vitamin B_6 0·2 mg., nicotinic acid 1 mg. and choline chloride 50 mg., appeared to be deficient in other respects, because the young were not successfully reared even when pantothenic acid was included at a level of 2·8 mg. per 100 g. of diet.

Nelson and Evans (1946) confirmed the finding of Jukes (1940) that rats deprived of pantothenic acid during gestation resorbed their foetuses instead of littering or produced poor litters. Two basal diets were used. When pantothenic acid was withheld from the diets for from 16 to 23 days before breeding, one-third of the rats failed to implant when mated with normal males. Of the remainder, 50 per cent. of implantations underwent resorption on one basal diet, 100 per cent. on the other basal diet. Such litters as were produced were small in number and in average birthweight compared with those of control does which reproduced normally on the basal diets with a supplement of 2·8 or 5·0 mg. Ca pantothenate per 100 g. diet. That the effect on reproduction was due to lack of pantothenic acid *per se* and not to reduced intake of food in the deficient group was demonstrated by means of paired feeding tests. Deprivation of pantothenic acid from the 13th day of gestation did not adversely affect the number of young or their average weight at birth but the percentage of young reared to 21 days of age was only 23 on one basal diet, none on the other basal diet. None of the young whose mothers had been deprived of pantothenic acid throughout gestation survived to 21 days. Over 80 per cent. of the young survived when deprivation dated from the beginning of lactation. In every instance the effect of deprivation of pantothenic acid was greater on one basal diet which differed from the other only in containing an improved salt mixture and larger amounts of the other members of the B complex.

Reference has already been made to the observation of Kline *et al.* (1938) that omission of pantothenic acid from a purified diet given to female rats from about parturition resulted in sudden respiratory failure in the young with collapse and death between the 10th and 20th day.

Sure (1945) in continuation of the experiment described above included vitamin B_6 in the purified diet in the same amounts as vitamin B_1, omitted pantothenic acid and included 15 per cent. of rice polishings as source of pantothenic acid. Young were not reared. The estimated amount of pantothenic acid supplied daily on the basis of a 20 g. food intake was 277·5 μg. When this was supplemented by 300 μg. Ca pantothenate, furnishing an additional 253 μg. pantothenic acid, the percentage reared was 50. A similar result was obtained with rice bran as source of pantothenic acid. Since the highest percentage of young reared in this investigation was 68 per cent. Sure considered that the 32 per cent. mortality unaccounted for might be due to some missing dietary factors or to imbalance of other essential nutrients in the rations employed.

Comparison of Different Levels of Pantothenic Acid in the Diet

Breeding Performance

Rats

Unna *et al.* (1941) succeeded in rearing black rats through 3 generations on a basal diet of dextrose 68, purified casein 18, crisco 8, salt mixture 4, cod liver oil 2, supplemented with 0·8 mg. each of vitamin B_1, riboflavin and vitamin B_6, 10 mg. nicotinamide, 100 mg. choline chloride and 100 μg. pantothenic acid per 100 g. of diet. The level of pantothenic acid was adequate to prevent achromotrichia. This estimate coincides with that of the requirement of the growing rat for Ca pantothenate obtained by Henderson *et al.* (1942) by means of balance studies on black or piebald rats.

Henderson *et al.* (1942) reared 4 generations of black or piebald rats on a ration consisting of sucrose 73, purified casein 18, salts 4 and maize oil 4 per cent., supplemented by halibut liver oil, α-tocopherol, and vitamin B_1 0·2 mg., riboflavin 0·3 mg., vitamin B_6 0·2 mg., nicotinic acid 0·25 mg., choline 100 mg. and Ca pantothenate 2 mg. per 100 g. of ration, but some mothers were not successful in rearing their young. This may have been due in part to deficiency of cystine.

Taylor *et al.* (1943) reported that when 5-month-old female albino rats maintained on a diet of purina dog chow containing 14 μg. pantothenic acid per g., were given a supplement of 100 μg. Ca pantothenate per 100 g. of ration and mated 2 weeks later, they produced litters which were 27 per cent. larger than those produced by females which did not receive the extra supplement of pantothenic acid.

Restricted Supply of Choline

PREGNANCY AND LACTATION

Rats

Sure (1940) reported that when does were transferred from a stock diet at parturition to a diet of purified casein 25, cystine 0·2 or 0·5, butterfat 10, salts 4, agar agar 2 per cent. and dextrin, supplemented with 120 μg. vitamin B_1, 120 μg. riboflavin, 50 μg. vitamin B_6, 15 mg. choline chloride, 1 ml. of Frost and Elvehjem's W factor and 6 mg. nicotinic acid, the weight increase of the nursing young was comparable to that obtained with does on a stock diet. Tests in which members of the supplement were omitted singly and supplied in graded amounts during lactation until the desired growth rate of the young was achieved, showed that the intake of vitamin B_1 had to be stepped up to 120 μg. during lactation to give the stock rate of liveweight increase. The necessary levels of riboflavin, vitamin B_6 and choline chloride were 120 μg., 50 μg. and 15 mg., respectively. In some cases normal weight increase of the nursing young in the vitamin B_1, riboflavin and choline tests was achieved only when a large proportion of the supplement was given direct to the young. When choline chloride was withheld from the diet of the doe paralysis occurred in the young. This could be cured by direct administration of a total of 15 mg. choline chloride to the litter ; otherwise death ensued between the 15th and 18th day of lactation. It should be clearly understood that these " requirements for lactation " do not necessarily bear any quantitative relationship to the amounts which, supplied to female rats from weaning, would suffice for optimum reproduction and rearing of young.

Sure's findings with regard to choline are not in agreement with those of Jacobi and Baumann (1942). In the course of an investigation of the effect of choline deficiency on growing rats these workers observed that 2 females on the choline-free diet produced and reared litters which increased in weight by 14 and 19 g., respectively, during the first 3 weeks of life, compared with 18 g. for the litter of a female getting 50 mg. choline daily. The choline-free diet used consisted of sucrose 65, purified casein 25, maize oil 5 and wesson salts 5 per

cent., supplemented with carotene, calciferol, vitamin B_1, riboflavin, vitamin B_6, nicotinic acid and pantothenic acid.

Peet and Sampson (1948) recently reported lack of contractility and partial atrophy of the uterus of female rats given a high-fat, choline-deficient diet for 39 days from parturition, but no paralysis in the litters.

Jervis (1942), however, confirmed Sure's observations. A group of 5 female rats from about the 8th day of pregnancy was given a diet low in choline and high in cystine. The diet consisted of casein 25, cystine 0·5, dextrin 58·5, agar 2, crisco 10 and salt mixture 4 per cent., supplemented by 100 μg. vitamin B_1, riboflavin and vitamin B_6 with 6 mg. nicotinic acid daily and 2 drops of halibut liver oil weekly. Two control females received in addition 100 mg. choline per 100 g. of food. No significant difference in the young was observed until the 10th to 12th day when growth stopped in the choline-deficient group. At this stage about 50 per cent. of the young in this group died. Those which survived developed paralysis of the limbs accompanied by convulsive movements. Death followed in 3 to 5 days. Post mortem examination revealed haemorrhagic lesions of the cerebellum. As early as 1927 Moore *et al.* (1927) noted the occurrence of brain haemorrhages in the young of rats reared on a diet deficient in the B vitamins. The observations of Brown *et al.* (1947) on the findings of Moore *et al.* and of Jervis have been referred to already (page 63).

The difference between the findings of Sure (1940) and Jervis (1942) on the one hand and Jacobi and Baumann (1942) and Peet and Sampson (1948), may be due to the fact that the former supplemented the casein in the diet with cystine. Mulford and Griffith (1942) showed that purified choline-deficient diets containing less than 30 per cent. of casein as source of protein did not contain enough methionine to make up for lack of choline. (For literature on the role of methionine as a donator of methyl groups in the synthesis of choline *in vivo*, see the recent review by Best and Lucas (1945).) Mulford and Griffith found also that addition of cystine to such diets increased the deposition of liver fat and the incidence of renal lesions in young rats ; in other words, increased the choline requirement. Admittedly, it is not known if the same argument would apply to the maternal requirement of choline for successful rearing of the young.

Mice

Recently White and Cerecedo (1946) studied the effect of choline on reproduction and lactation in mice. Albino mice were weaned to a diet of purified casein 25, sucrose 53, crisco 10, lard 5, salts 5 and ruffex (α-cellulose) 2 per cent., supplemented with 10 mg. of vitamin B_1, riboflavin and vitamin B_6, 100 mg. Ca pantothenate, 5000 I.U. vitamin D, 40 mg. α-tocopherol and 2 mg. β-carotene per kg. of diet. On this diet 23 per cent. of the litters produced were weaned. When the diet was supplemented with 1·5 g. choline per kg. diet the percentage of litters weaned was increased to 36. Further addition of a folic acid concentrate allowed 50 per cent. of the litters to be weaned. When the casein content of the basal diet was increased to 33 per cent. at the expense of sucrose there was no beneficial effect on the number of young reared. When this diet was supplemented with choline, however, 55 per cent. of the litters were weaned and further addition of folic acid increased the percentage to 65.

Syrian hamsters

Hamilton and Hogan (1944) reported an adverse effect on the rearing of young when choline was omitted from a purified diet for breeding hamsters. Details of the diet are given on page 86.

Effects of Supplements of *p*-Aminobenzoic Acid and Inositol to Purified Diets

Rats

Sure (1941*b*, *c* ; 1942) reared female rats from weaning on a purified basal diet containing as source of protein 20 per cent. of blood fibrin or 20 per cent. of casein plus 0·3 per cent. of cystine or 10 per cent. each of fibrin and casein plus 0·2 per cent. of cystine and suitably

supplied with fat-soluble vitamins and nicotinic acid. The diet was supplemented also with 20 μg. each of vitamin B_1, riboflavin and vitamin B_6, 6 mg. choline chloride and some W factor; during pregnancy the amounts of these B complex components were increased 1·5-fold and during lactation 2·5- to 6-fold. The does failed to rear their litters. Large supplements of Ca pantothenate during pregnancy and lactation were ineffective, but the young were reared when a rice-bran extract or a liver extract was added at the critical point during lactation. At this stage of the work the high percentage of sterility and of stillbirths in the experimental animals led to a change being made in the salt mixture. The Ca : P ratio, which had been 4 : 1, was improved and the Mn content was increased. This resulted in greatly improved reproduction, but no improvement in rearing. Addition of *p*-aminobenzoic acid with or without inositol to the purified diet enabled young to be reared. In a subsequent investigation (Sure, 1943) it was pointed out that the unknown lactation factor in rice-bran extract, liver extract and yeast, designated B_x, might consist of more than one component. In order to ensure a response to *p*-aminobenzoic acid, therefore, various small supplements were added to the basal diet, namely, 2 per cent. of dried grass, 1 per cent. of liver extract, 2 per cent. of dried liver or 0·2 per cent. of yeast nucleic acid. Addition of *p*-aminobenzoic acid resulted in a marked increase in the percentage of young weaned. Inositol was found to have a depressing effect when given alone, but not when given along with *p*-aminobenzoic acid. In the course of this investigation further changes were made in the basal diet. Blood fibrin at a level of 25 per cent. was used as source of protein, dextrose replaced dextrin as carbohydrate and the salt mixture was given at a level of 5 per cent. Rats reared on this new basal diet supplemented with the known essential vitamins (but not with a source of the B_x factor) were able to rear 40 per cent. of the young they were allowed to suckle, compared with only 6 per cent. when the protein was supplied as 20 per cent. of fibrin or 10 per cent. of fibrin and 10 per cent. of casein fortified with cystine, the carbohydrate as dextrin and the same salt mixture at a level of 4 per cent. When part of the glucose in the new basal ration was replaced by polished rice at a level of 30 per cent. in the diet, female rats reared and bred on the diet were able to rear 66·6 per cent. of their young. With this group as control the effect of including 0·2 per cent. of *p*-aminobenzoic acid and or 0·2 per cent. of inositol in the diet of does from weaning was studied. The results are shown in Table XX.

TABLE XX

Effect of Inositol and *p*-Aminobenzoic Acid on Reproduction and Rearing of Young Rats

Supplement to Basal Diet (which included 30 per cent. of polished rice)	No. of Females Mated	Young		
		No. of Litters	Total No. Born	Per cent. Reared of those Allowed to Suckle
None	5	11	96	66·6
0·2 per cent. of inositol	5	12	112	31·3
0·2 per cent. of *p*-aminobenzoic acid	5	11	112	98·4
0·2 per cent. of inositol and 0·2 per cent. of *p*-aminobenzoic acid	5	10	108	88·3

The harmful effect of inositol is discussed in relation to Martin's (1942) findings that administration of 2 mg. inositol daily to black rats on a purified diet supplemented with vitamin B_1, riboflavin, vitamin B_6, nicotinic acid, pantothenic acid and choline chloride resulted in poor weight increase and changes in the coat which could be prevented if 1 mg.

p-aminobenzoic acid was also given daily. Sure, however, observed no such syndrome in the albino litters of his lactation experiment nor in growing albino rats given 10 mg. inositol daily.

Sure's (1943) findings are at variance with those of Climenko and McChesney (1942). The latter reared young female rats from weaning on the purified basal diet used by Sure in 1940 with a supplement of the known components of the B complex, consisting of 120 μg. each of vitamin B_1, riboflavin and vitamin B_6, 600 μg. Ca pantothenate, 6 mg. nicotinic acid and 15 mg. choline chloride, the amounts usually provided by Sure during lactation. Other groups received this diet supplemented with 15 mg. *p*-aminobenzoic acid and/or 15 mg. inositol daily. A group on a stock ration acted as normal controls. The experimental rats were mated at a weight of about 180 g. with a male of proved fertility. Litter weights obtained daily from birth were used as an index of milk production. These showed that in the absence of *p*-aminobenzoic acid and inositol, lactation was slow of initiation and ceased about the 14th day. Inclusion of a *p*-aminobenzoic acid supplement appeared to delay the initiation of lactation until about the 5th day, after which lactation was normal. The inositol supplement resulted in prompt initiation of lactation which was maintained at a level slightly below that of normal controls. Combined supplementation with *p*-aminobenzoic acid and inositol gave no significant improvement in lactation over that obtained with inositol alone. The cumulative mortality of the young in each group during the first 15 days of life was shown graphically. In the group receiving neither *p*-aminobenzoic acid nor inositol the mortality was a little over 50 per cent. by the 14th day. In the group receiving *p*-aminobenzoic acid the mortality reached a plateau of about 40 per cent. by the 6th day. In the group receiving inositol mortality reached a plateau of 20 per cent. by the 9th day. Addition of both supplements resulted in a slight improvement in mortality rate as compared with inositol alone, so that the mortality rate in this group, which reached a plateau of just over 10 per cent. by the 5th day, was about the same as for the normal controls.

In this connection the observations of Ershoff (1943) and Ershoff and McWilliams (1943) are of interest. Albino rats reared on a diet of purified casein 22, sucrose 73·2, cystine 0·3 and salt mixture 4·5 per cent., supplemented per 100 g. of diet by 500 μg. vitamin B_1 and vitamin B_6, 1 mg. riboflavin, 10 mg. nicotinic acid and pantothenic acid, 15 mg. *p*-aminobenzoic acid, 0·1 g. choline chloride and 40 mg. of inositol, showed a peculiar tail dermatitis distinct from that caused by deficiency of pantothenic acid, vitamin B_6 or essential fatty acids. Out of 7 males raised on this diet, 5 mated successfully the first time they were placed with normal oestrous females. Females raised from weaning on the synthetic diet showed regular oestrous cycles and when 4 were mated with normal males of proved fertility they produced litters of 8 or 11 normal young. In no instance, however, did the mother nurse her young. In another group of rats reared from weaning on this diet with the inositol omitted, dermatitis was not observed (Ershoff, 1943).

When inositol was omitted from the diet and a supplement of 0·5 g. sulphaguanidine was given, females reared on the diet reproduced normally and reared 3 out of 8 litters. When both inositol and sulphaguanidine were given only 2 out of 9 rats produced litters and lactation was not observed. Controls on a diet of natural foods showed normal reproduction and rearing of young (Ershoff and McWilliams, 1943).

Later Ershoff (1946*b*) studied the effects of massive doses of *p*-aminobenzoic acid and inositol on reproduction in the rat. A group of female rats of the Long-Evans strain was given a basal diet (*a*) containing casein 30, salt mixture 4·5 and sucrose 65·5 per cent. Other groups were given the basal diet with the following inclusions in place of equivalent amounts of sucrose : (*b*) 1·0 per cent. of *p*-aminobenzoic acid ; (*c*) 2·0 per cent. of *p*-aminobenzoic acid ;

(*d*) 1·0 per cent. of inositol; (*e*) 1·0 per cent. of *p*-aminobenzoic acid and 1·0 per cent. of inositol; (*f*) 0·0005 per cent. of folic acid; (*g*) 10 per cent. of yeast. To all of the diets except the last were added per kg. 80 mg. vitamin B_1 hydrochloride, 20 mg. riboflavin, 20 mg. vitamin B_6 hydrochloride, 100 mg. Ca pantothenate, 100 mg. nicotinic acid, 5 mg. 2-methyl-naphthaquinone and 1·2 g. choline chloride. The last 2 supplements were also added to diet (*g*). In addition all rats received 3 times weekly 500 mg. cottonseed oil, 1·0 mg. α-tocopherol and a vitamin A and D concentrate. After 60 days on the diets 6 females of each group were mated with males of proved fertility. There was no difference in reproduction between the groups. The average birthweight was 5 to 5½ g. in all groups. Litters were reduced to 6 at birth. Young were nursed and weaned in all groups except (*e*) and (*f*). In the basal diet group (*a*) 2 litters were nursed but only 5 of the 12 young survived to weaning at an average weight of 22·8 g. In each of the groups given 1·0 per cent. of *p*-aminobenzoic acid (*b*), 1·0 per cent. of inositol (*d*) and 10 per cent. of yeast (*g*) 3 mothers successfully nursed their young which were weaned at 21 days at average weights of 36 to 41 g. Only 1 of the does on the diet containing 2·0 per cent. of *p*-aminobenzoic acid reared a litter to weaning and of this litter there were only 3 survivors at weaning of an average weight of 24·6 g.

The cause of the poor rearing of young was not determined, but Ershoff concluded that 1 per cent. of *p*-aminobenzoic acid or inositol had no adverse effect on reproduction.

A further contribution to the problem was made by the findings of Richardson and Hogan (1945), of which only a brief abstract has appeared. Female rats were given a purified ration containing per 100 g. 2000 I.U. vitamin A, 283 I.U. vitamin D, 2·5 mg. α-tocopherol, 2·5 mg. vitamin K, 1 mg. each of vitamin B_1, riboflavin and vitamin B_6, 2 mg. Ca pantothenate, 200 mg. choline, 5 mg. nicotinic acid, 100 mg. inositol, 50 mg. *p*-aminobenzoic acid and 20 mg. biotin. A total of 60 litters from females on this diet was obtained. Seventy per cent. of the litters and 69·7 per cent. of the young were weaned. When inositol was omitted, 44·3 per cent. of the young were weaned, when nicotinic acid was omitted 60·4 per cent. and when *p*-aminobenzoic acid was omitted 75·8 per cent. Richardson and Hogan's results confirm the findings of Climenko and McChesney (1942) that inositol has a beneficial effect on lactation. The results obtained with inositol in the diet, and *p*-aminobenzoic acid omitted, were slightly better than when both of these vitamins were included. This is not in agreement with the results of either Sure (1943) or Climenko and McChesney (1942). Addition of 1 per cent. of fuller's earth eluate of liver which was stated to supply vitamin B_c (now identified with folic acid) and probably other unrecognised vitamins, increased the weaning percentage to 83·4. Since a liver filtrate which contained only 0·3 μg. vitamin B_c was equally effective, Richardson and Hogan concluded that there is an unrecognised nutrient which is essential in the diet if female rats are to wean the maximum number of young.

Syrian hamsters

Hamilton and Hogan (1944) succeeded in rearing hamsters from weaning on a purified diet containing inositol and *p* aminobenzoic acid and the females produced at least 1 litter. When inositol was omitted few females bore living young. The results of withdrawing *p*-aminobenzoic acid were inconclusive. Details of the diet are reported on page 86.

Restricted Supply of Nicotinic Acid

Rats

Richardson and Hogan (1945) reported a beneficial effect on the rearing of young rats when nicotinic acid was included in the diet, which was not found by Sure (1940) but has been suggested by Rutto and Vescia (1943). Ershoff (1946*a*) recently obtained successful reproduction and rearing of young by albino rats which received from weaning a synthetic diet lacking in nicotinic acid and which were mated with fertile males. The basal diet of

purified casein 22, salt mixture 4·5 and sucrose 73·5 per cent., was supplemented daily with 500 mg. maize oil, 0·5 mg. α-tocopherol, and a vitamin A and D concentrate containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D. Approximately half of the rats were given the following amounts of vitamins in mg. per 100 g. of ration: vitamin B_1 hydrochloride 0·2, riboflavin 0·4, vitamin B_6 0·2, Ca pantothenate 3·0, choline chloride 120·0 and vitamin K 0·2. The others received these vitamins respectively in the following amounts in mg. per 100 g. of ration: 20·0, 40·0, 2·0, 20·0, 120·0 and 0·5. Male albino rats on these diets were fertile when mated with normal females. Female hooded rats of the Long-Evans strain were reared on the synthetic diets. No significant difference was observed in the ability of the 2 strains to reproduce on these diets. Of the total of 29 females bred, 27 produced litters, in 24 of which all the young were born alive, with an average of 9·4 young per litter. The average birthweight was 5·6 g. Of the 15 albino litters, 9 were nursed to weaning. Litters were reduced to 6 at birth and 39 out of 44 young (89 per cent.) were successfully weaned at 21 days, with an average weight of 31·9 g. Mothers of the Long-Evans strain failed to nurse their young. Ershoff points out that this result does not necessarily indicate a strain difference in nicotinic acid requirement for lactation; it might be due to a strain difference in the synthesis or requirement of inositol, *p*-aminobenzoic acid, biotin, or folic acid, none of which was added to the basal diet. Nelson and Evans (1947*a*) also have reported the absence of any beneficial effect on reproduction and lactation in rats of the Long-Evans strain when nicotinic acid was included in a purified diet.

Syrian hamsters

Hamilton and Hogan (1944) reported an adverse effect on the number of young reared by female hamsters given a purified diet from which nicotinic acid was omitted. Details of the diet are reported on page 86.

Effects of Supplements of Folic Acid, Biotin and Liver

"Folic acid" was the name given in 1944 to a material obtained in almost pure form from spinach, which supported the growth of *Lactobacillus casei* and *Streptococcus faecalis*. Different forms of "folic acid" have been obtained from liver, yeast, and other sources. Liver *L. casei* factor was synthesised in 1946 and its structure is that of pteroylglutamic acid. In the following pages the terms used by the authors themselves have been adhered to.

Rats

Morse and Schmidt (1944) compared the nitrogen balances of female rats during pregnancy and lactation on a diet of purified casein, cane sugar, salts, crisco and maize oil, supplemented with all the usual vitamins (but not biotin or folic acid), with those of rats on the same diet supplemented with methionine or liver extract. Under the conditions of the experiment the diet supplemented with liver extract compared most favourably with a stock ration in preventing loss of nitrogen in pregnancy and lactation. It was suggested, although direct proof was lacking, that this might indicate the presence of one or more factors in liver extract required by rats during pregnancy and lactation.

Richardson and Hogan (1946) described the occurrence of hydrocephalus in suckling rats whose mothers received a synthetic diet consisting of casein 30, cerelose (glucose) 52, wood pulp 3, lard 10, and salts 5 per cent., with fat-soluble vitamins and members of the B complex. The B vitamins were given in the amounts described in a previous paper (Richardson and Hogan, 1945), except that the supplement of Ca pantothenate was doubled and *p*-aminobenzoic acid and choline were each given at a level of 100 mg. per 100 g. of ration. The number of female rats on this diet was 230; in all 1756 young were reared to weaning at 28 days, but of 30 young which developed hydrocephalus between 10 and 24 days of age only 2 survived as long as 28 days. Another group of 54 female rats was given a supplement

of an eluate of a fuller's earth adsorbate of a liver extract. In this group a total of 1020 young survived to weaning with no indication of hydrocephalus. Nor were there any hydrocephalic young among several thousand stock colony young. Richardson and Hogan concluded that the abnormality was due to a deficiency of an unidentified nutrient which is present in the liver eluate and in many natural foods.

Rombouts and Querido (1946) reported satisfactory reproduction in 7 female rats maintained on a synthetic diet from weaning. The diet consisted of sucrose 74, vitamin-free casein 18, cottonseed oil 2, cod liver oil 2 and salt mixture 4 per cent., supplemented in mg. per kg. with vitamin B_1 hydrochloride 2, riboflavin 2·5, vitamin B_6 hydrochloride 3, Na pantothenate 10, nicotinic acid 300 and choline chloride 300. But 65 per cent. of the young died before weaning. The average weaning weight at 28 days was 28 g.

Vinson and Cerecedo (1944) presented data on reproduction and lactation for 4 generations of rats on a diet of purified casein 30, salts 5, ruffex (α-cellulose) 2, lard 5, crisco 10, and sucrose 48 parts, supplemented per kg. of diet by 20 mg. each of vitamin B_1, riboflavin, vitamin B_6 and α tocopherol, 40 mg. each of Ca pantothenate and vitamin A and D concentrate and 500 mg. choline chloride. The number of young weaned was significantly less than on a diet of purina dog chow, and the mothers showed a sharp loss of weight during lactation. Addition of biotin, *p*-aminobenzoic acid, inositol and yeast nucleic acid failed to prevent the loss of weight of the mothers. Addition of folic acid or *Lactobacillus casei* factor to the purified diet from weaning or from mating improved the lactation performance as shown in Table XXI (Cerecedo and Vinson, 1944*b*).

TABLE XXI

Effect of Folic Acid Supplement to Purified Diets on Lactation Performance of Rats

Supplement per kg. Basal Diet	No. of Litters	Av. Litter Size	Av. Weaning Weight (g.)	Av. Weight Loss (−) or Gain (+) of Mothers during Lactation (g.)
None. Basal diet from weaning . . .	2	4	28	−19·0
25 mg. folic acid (1) concentrate from weaning .	3	5	43	+ 9·0
5 mg. folic acid (2) concentrate from mating .	2	8	34	−15·0
2·5 mg. *L. casei* factor (3) from mating . .	2	7	39	+ 3·0

Relative folic acid potency of concentrates (1) 5000 (2) 30,000 (3) 60,000

Folley *et al.* (1947) failed to confirm these observations. They used a purified basal diet containing per cent. of dry diet sucrose 60, purified casein 20, lard 3, groundnut oil 12 and salts 5, supplemented by vitamins per rat daily as follows: in μg., vitamin B_1 15, riboflavin 50, vitamin B_6 10, Ca pantothenate 300; and in mg., choline hydrochloride 3, nicotinic acid 1, inositol 1, *p*-aminobenzoic acid 3. A supplement of 2 mg. vitamin K dissolved in 40 mg. cod liver oil was given each day except on one day of the week when 2 mg. α-tocopherol acetate dissolved in arachis oil were given. During pregnancy the daily intake of riboflavin, nicotinic acid, inositol and choline was doubled and that of vitamins B_1 and B_6 trebled. The two latter were further increased to 120 and 50 μg., respectively, during lactation. A supplement of cystine, 0·2 per cent. of the dry diet, was included. This diet was given, alone or supplemented, to groups of black-and-white female rats from weaning. The general procedure was to mate experimental females with stock colony male rats. Litters were reduced to 8 at birth, females being kept in preference to males. Mothers were killed when they had reared one litter or had twice failed to do so. On the basal diet alone 5 generations

were reared but the production and rearing of young was poor compared with that of control rats on a stock diet, and deteriorated in successive generations. Supplements of milk, whole liver extract and of a biotin concentrate gave somewhat better results and whole liver better still. A supplement of arginine, histidine, additional cystine, pure biotin 1 μg. daily and pure folic acid 10 μg. with double these amounts of biotin and folic acid during pregnancy and lactation was ineffective. The results are shown in Table XXII.

TABLE XXII

PRODUCTION AND REARING OF YOUNG BY RATS ON PURIFIED DIET

Diet of Mother	No. Litters (1st and 2nd)	Young				
		Av. No. Live Young per Litter	Percentage Reared	Av. No. Weaned per Litter	Av. Weaning Age (days)	Av. Weaning Weight (g.)
Stock . . .	16	10·6	91·4	7·7	21	44·5
Basal . . .	16	7·5	50·0	5·4	27·8	26·5
Basal + suppl. of amino-acids, biotin and folic acid .	16	7·3	50·0	5·7	27·1	29·2

In all groups except the one receiving liver, the mothers lost weight during lactation. Folley *et al.* concluded that the basal diet lacked some factor or factors necessary for normal reproduction and lactation, present in fresh liver, liver extracts, milk and the biotin concentrate used, but that pure biotin and pure folic acid were not beneficial.

Nelson *et al.* (1946) stated that rats maintained throughout their entire life on a synthetic diet have weaned 90 per cent. of their young, which was equivalent to the lactation performance of stock animals in their rat colony. The basal diet consisted of alcohol-extracted casein 24, sucrose 64, hydrogenated vegetable oil (crisco) 8, and salt mixture 4 per cent. Crystalline B vitamins were added per kg. of diet as follows: vitamin B_1 hydrochloride 5 mg., vitamin B_6 hydrochloride 5 mg., riboflavin 10 mg., *p*-aminobenzoic acid 10 mg., nicotinic acid 20 mg., Ca pantothenate 50 mg., inositol 400 mg. and choline chloride 1 g. One ml. of a fat-soluble vitamin mixture containing 6 mg. α-tocopherol, 115 chick units of vitamin D, 800 U.S.P. units vitamin A and 650 mg. maize oil was given weekly to each litter.

Although the synthetic diet was adequate for successful reproduction and rearing of young, adult female rats maintained on this diet from parturition showed a marked leucopenia and granulocytopenia by the 21st day of lactation, which were not observed in lactating females on stock diet. Daily supplements of brewer's yeast or an alcohol extract of liver resulted in improvement but did not entirely prevent the occurrence of the abnormal blood picture. Preliminary studies with a folic acid concentrate showed that 7.5 μg. folic acid daily during lactation had no beneficial effect. Nelson *et al.* concluded that either considerably higher levels of folic acid or additional factors must be necessary for a normal leucocyte picture in rats during lactation.

In a subsequent investigation in which the same basal diet (except for addition of 2-methyl-1 : 4-naphthaquinone) was used Nelson and Evans (1947*c*) found that both higher levels of synthetic pteroylglutamic acid and an additional unknown factor(s) present in liver eluate powder appeared to be necessary. The results obtained when the basal diet alone and supplemented with synthetic pteroylglutamic acid or liver eluate powder was given to rats of the Long-Evans strain from parturition compared with those obtained on a stock ration

are shown in Table XXIII. Inclusion of 110 μg. synthetic pteroylglutamic acid per 100 g. of purified diet from parturition had a beneficial effect on the weight change of the mother and on total leucocytes and the percentage of granulocytes in the mother's blood at weaning which was not improved by further increases in the amount of the supplement. Addition of 275 μg. of the supplement resulted in slightly higher average weaning weights of young. Supplements of liver eluate powder supplying these amounts of pteroylglutamic acid were effective also but the effect on maternal leucocyte count was much greater, the count approaching closely that of stock females when the liver powder was included at the highest level tested (1 per cent.).

Sporn *et al.* (1947) obtained a higher rate of survival of suckling rats of the Sprague-Dawley strain whose mothers were given from weaning a purified basal diet supplemented with 0·5 g. fresh liver daily than in those whose mothers received no supplement to the basal diet. The basal diet consisted of sucrose 73, casein 18, maize oil 5, salts 4 per cent., with in mg. per 100 g. of ration: vitamin B_1 0·3, riboflavin 0·3, nicotinic acid 2, vitamin B_6 0·2, pantothenic acid 2, crystalline folic acid 0·025, crystalline biotin 0·01, inositol 10, choline 100 and *p*-aminobenzoic acid 25. Vitamins A and D were given twice weekly in maize oil and a single dose of vitamin E immediately before mating with stock male rats. Litters were reduced to 6 at birth. The percentage of young surviving to weaning at 21 days was 65 in the supplemented group compared with 73 in the unsupplemented group. The corresponding percentage survival in a third group given a supplement of 5 ml. raw milk daily was 66. These are the combined results of first and second litters. Sporn *et al.* concluded that liver contains a factor necessary for "maximum survival of young."

The need to add biotin to purified diets for breeding rats has not been shown, possibly because diets free from biotin are difficult to obtain, but biotin deficiency can be produced in rats by the inclusion in the diet of raw egg white, the avidin of which can apparently combine with biotin to render it unavailable to the rat. As early as 1930 Clayton observed that reproduction in a group of 8 females and 3 male rats on a diet of starch, salt, yeast, cod liver oil and 15 per cent. of raw egg white was poor and none of the females succeeded in rearing young. Recently Kennedy and Palmer (1945) investigated the effect of biotin deficiency on reproduction and lactation in the rat. Virgin rats aged 90 to 100 days were given a diet of uncoagulated egg albumin 30, lard 5, salts 4, cod liver oil 2, wheat germ oil 0·8, choline chloride 0·1 and sucrose 58·1 per cent., supplemented by 80 μg. vitamin B_1, 160 μg. riboflavin, 120 μg. vitamin B_6 and 100 μg. Ca pantothenate daily. Some were given daily supplements of 2 or 6 μg. biotin. Black rats getting no biotin or 2 μg. succeeded in rearing their first litters, but albino rats failed to do so. When the rats were mated a second time implantation occurred but there was frequent early resorption of the foetus. Even with 6 μg. biotin daily, reproduction and rearing of young were not satisfactory, and replacement of half of the raw egg white by casein did not effect any improvement. It was concluded that biotin is necessary for successful gestation and the birth of young and is probably necessary for lactation, although the inadequacy of the diet in other respects (it is pointed out that folic acid was not added) prevented the effect of biotin on lactation being shown.

Rats

The existence of unidentified factors essential for lactation in the rat has been postulated by Nakahara and his associates. In 1933 Nakahara and Inukai reported that a diet of polished rice powder 75, fish protein 10, butter 10, salt mixture 5 and dried yeast 5 parts was adequate for growth, pregnancy and parturition in rats but failed to support lactation. Later they described the preparation from an acid alcohol extract of liver of a factor L which had to be added to their basal diet; without it almost complete failure of lactation occurred. They

TABLE XXIII

EFFECTS OF PTEROYLGLUTAMIC ACID AND LIVER ELUATE ON LACTATION PERFORMANCE AND BLOOD PICTURE OF FEMALE RATS

Supplement per 100 g. Purified Diet	No. Litters	Av. Weaning Wt. of Young g.	Per Cent. Weaned	Wt. Change of Mother During Lactation g.	Av. Daily Food Consumption During Lactation g.	Av. Daily Intake of Pteroyl-glutamic Acid µg.	Maternal Blood at Weaning	
							Leucocytes per c.mm.	Per Cent. Granulocytes
None	70	42 ♂ 40 ♀	95	−26·0	27·8	—	4350	18
110 µg. pteroylglutamic acid	26	42 ♂ 42 ♀		+8·6		30	7545	32
275 µg. „ „	23	46 ♂ 43 ♀	95	+6·3	29·9	81	7650	29
550 µg. „ „	24	46 ♂ 44 ♀		+7·1		165	7300	29
0·2 per cent. liver eluate powder (110 µg. pteroylglutamic acid)	35	47 ♂ 46 ♀		+2·5		36	7995	32
0·5 per cent. liver eluate powder (275 µg. pteroylglutamic acid)	36	49 ♂ 48 ♀	98	+16·2	32·9	92	9810	30
1 per cent. liver eluate powder (550 µg. pteroylglutamic acid)	35	50 ♂ 49 ♀		+10·3		181	10,245	28
Stock diet + lettuce	70	50 ♂ 48 ♀	96	+19·6	36·3	—	10,850	30

considered that this factor L, since it was not adsorbed on acid earth, was different from vitamin B (Nakahara *et al.*, 1935). Since then vitamin B has been shown to be a complex which includes filtrate factors which are not adsorbed by fuller's earth. Later they postulated the existence of a second factor L_2 in an acid alcohol extract of yeast which could be prepared in the same way as the liver factor, which they now designated L_1 (Nakahara *et al.*, 1936). Nakahara and his associates continued their investigations into the L complex (Nakahara *et al.*, 1938; 1939; Ugami, 1938) and reported (Nakahara *et al.*, 1939) that the effects of deprivation of vitamin L were less severe in mice than in rats. Mice were still able to rear about 20 per cent. of their young after complete deprivation of vitamin L for 87 to 98 days, while rats reared none after about 35 days' deprivation. Mice

Folley *et al.* (1938) attempted to repeat Nakahara's experiments, but found that female rats were able to rear between 45 and 65 per cent. of their young when fed from weaning on a diet much the same as that upon which the Japanese workers had observed almost complete failure of lactation. These workers, however, used brewer's yeast, and Nakahara *et al.* (1939) later described this as a good source of their L factors. Rats

The Japanese workers recently turned their attention to the filtrate factors of the B_2 complex (Nakahara *et al.*, 1940; 1941*a*, *b*). They reported that synthetic pantothenic acid was not capable of replacing factor L_1 or L_2, and were uncertain whether Elvehjem's factor W was a lactation factor. They expressed the view that none of the filtrate factors of the vitamin B_2 complex is identical with the L factors. Yeast nucleic acid was suggested as a possible component of factor L_2.

Ball and Barnes (1941) studied two successive pregnancies in groups of mice reared on a purified basal diet of casein 31, glucose 34, lard 20, yeast 8 and salts 7 per cent., supplemented with cod liver oil and wheat germ oil. On this diet only 3 per cent. of the young were weaned. The percentage weaned was increased in groups receiving additional supplements of dried grass or bran, but even with both supplements only about 40 per cent. of the young were weaned. Addition of ascorbic acid, liver extract as source of factor W, or inositol had no beneficial effect. Ball and Barnes concluded that the lactation-promoting effect of the dried grass and wheat bran was not due to an increase of vitamin B_1, riboflavin, pantothenic acid, factor W or inositol. Mice

Foster *et al.* (1943) reared 4 generations of mice on a diet of purified fibrin 25, salt 4, regenerated cellulose 4 and glucose 67 per cent., supplemented by vitamins A, D, K, E, linoleic acid and the following amounts of the vitamin B complex components per 100 g. of diet: 1 mg. each of vitamin B_1 chloride, riboflavin, and vitamin B_6, 6 mg. each of Ca pantothenate, nicotinic acid and inositol, 15 mg. *p*-aminobenzoic acid and 60 mg. choline chloride. The addition of biotin was not considered to be necessary. The percentage of young weaned in successive matings and in successive generations was in every case significantly lower than the corresponding data obtained with stock animals. This was true even when 2 per cent. of liver extract was included as a supplement to the purified diet. The average weaning percentage for all generations and matings on the synthetic diet was 65, compared with 85 for control mice on a stock diet. Liveweight increase of the young of females on the synthetic diet was inferior to that of stock young during the suckling period, but those which survived to weaning on the synthetic diet gradually overtook the controls. Two possible interpretations suggested themselves to the authors, either that the synthetic diet was adequate for growth but deficient in some factor necessary for the production of an adequate milk supply in the lactating doe, or the diet was deficient in some essential factor which could be synthesised in the intestinal tract of the young mouse once its intestinal microflora was established, but not by the suckling mouse. In the latter case it is presumed that the doe is unable to

synthesise enough to supply a litter of young through the milk and requires an additional dietary source of the factor.

Cerecedo and Vinson (1944*a*) succeeded in raising 4 generations of mice on purified diets of the type used in their rat experiments, but the percentage of young weaned was below normal. Addition of a concentrate of folic acid to the purified basal diet referred to on p. 77 increased the percentage of young albino mice weaned from about 40 to 80, which compared favourably with the percentage weaned by does on stock diets. A strain of black mice failed to wean their young on the basal diet, and although the folic acid supplement effected an improvement, rearing performance was still much below normal for the strain. Other supplements given in addition to the basal diet in an effort to improve lactation, namely, biotin 0·05 μg., yeast nucleic acid 9 mg. or *p*-aminobenzoic acid plus inositol, 250 mg. each, per kg. of diet were ineffective, but 100 mg. of brewer's yeast daily improved lactation. The possibility that larger amounts of folic acid are needed or that some other factor or factors are required remains to be investigated. Similar results were obtained when the basal diet contained 25 instead of 30 per cent. of casein and the amounts of vitamins supplied per kg. of diet were as follows: vitamin B_1, riboflavin and vitamin B_6 10 mg. instead of 20 mg., Ca pantothenate 100 mg. instead of 40 mg., choline chloride 1·5 g. instead of 500 mg., α-tocopherol 40 mg. instead of 20 mg., carotene 2 mg. and vitamin D 125 μg. instead of 40 mg. of a vitamin A and D concentrate.

A subsequent experiment with mice, the strain is not given, (Cerecedo and Mirone, 1947) in which 10 mg. synthetic *L. casei* factor was added per kg. of the original basal diet showed that addition of the pure substance increased the percentage of litters weaned and permitted a greater gain in weight of the does during lactation. The average weight of the young at weaning was the same in both groups. Mirone and Cerecedo (1947*a*) investigated the growth and fertility of male mice on this basal diet and found that it compared favourably with that of male mice on a stock ration. The addition of supplements of folic acid or a liver eluate fraction to the basal diet had no effect on fertility. The mice used were from a colony of animals representing 3 different strains. Mirone and Cerecedo concluded that the basal diet was adequate for growth and fertility of the male mouse.

A strain difference in the reproductive performance of mice reared on purified diets was observed by Rogers *et al.* (1942). Black mice were reared on a purified diet consisting of casein, glucose, crisco, linoleic acid, salt mixture, agar, wheat germ oil, rice polish filtrate factor II, cod liver oil, vitamin B_1, riboflavin, vitamin B_6, nicotinic acid, Ca pantothenate, choline, *p*-aminobenzoic acid and inositol. When 3 females were mated all bred successfully, and the young from 2 of them were reared on the same diet and successfully bred at 69 days of age. The second generation of young was also weaned successfully. Attemps to duplicate the results with a different strain of mice were unsuccessful.

Reference has been made to the failure of a strain of black mice to rear their young when given a purified diet upon which mice of a different strain had reared 80 per cent. of their young (Cerecedo and Vinson, 1944*a*). It may be noted here that Copeland (1944) has reported a strain difference in the choline requirement of growing rats. Albino rats from the Wistar strain had a lower requirement than hooded rats from the Wisconsin strain on the same basal diet.

Fenton and Cowgill (1947) reported that female mice given from weaning a purified diet containing casein 23, dextrose 60, crisco 10, salts 5 and ruffex 2 per cent., supplemented per 100 g. of diet with cod liver oil 0·2 g., linoleic acid 0·27 g. and in mg., choline 150, *p*-aminobenzoic acid 100, inositol 100, vitamin B_1 0·5, vitamin B_6 0·5, riboflavin 1, nicotinic acid 1, Ca pantothenate 3 and α-tocopherol 6, failed completely to rear their young. The

fact that offspring of females on this diet were reared successfully by foster mothers on stock diets indicated that the problem was one of failure of lactation. A supplement of a biotin concentrate or of cystine had no beneficial effect but a supplement of folic acid alone or with the biotin concentrate resulted in some improvement. When the basal diet was modified to contain casein 30, dextrose 52·5, cystine 0·5 per cent., linoleic acid omitted, the other constituents remaining as before, and supplemented with biotin concentrate 0·02, folic acid 0·05 and 2-methyl 1 : 4 naphthaquinone 1 mg. per 100 g. of diet, rearing of young was more successful. Whether all or only some of these changes contributed to the improvement was not determined. Fenton and Cowgill anticipated that further modification of the diet would bring about further improvement in reproduction and lactation.

Although supplementing the basal diet with a biotin concentrate at the level employed did not effect any improvement in lactation, Fenton and Cowgill showed that the bodies of 14 young of does on the basal diet contained on the average 0·064 μg. biotin per g. of wet tissue (range 0·032 to 0·094 μg.) compared with an average of 0·172 μg. of biotin per g. (range 0·159 to 0·184 μg.) in the bodies of 6 young of does on stock rations.

Mirone and Cerecedo (1947*b*) found that when female mice were given from weaning the purified basal diet described on page 77 supplemented with 5 mg. xanthopterin per kg. of diet the percentage of young reared was 83 compared with 41 on the basal diet.

Addition of 200 μg. biotin per kg. of the basal diet had a beneficial effect on both reproduction and rearing of young. The results obtained with 3 different strains of mice are shown in Table XXIV.

TABLE XXIV

THE EFFECT OF XANTHOPTERIN, PTEROYLGLUTAMIC ACID AND BIOTIN ON REPRODUCTION AND REARING OF YOUNG IN MICE

Supplement per kg. Basal Diet	No. Animals Bred	Av. Size Litter at Birth	Per Cent. Young Weaned	Av. Weight at Weaning (g.)
None	56	5·4	41	7·6
5 mg. xanthopterin	24	5·8	83	8·0
20 mg. pteroylglutamic acid	127	6·4	68	8·6
200 μg. biotin	9	6·4	75	8·8
Pteroylglutamic acid + biotin	36	7·2	83	9·5
Stock diet	82	6·7	95	9·0

The existence of an unidentified factor or factors essential for optimal performance of female rats and mice in the rearing of young has been postulated. The degree of success in the rearing of young obtained by different investigators with rats and mice on purified diets without the addition of any source of "unknown" factors is shown in Table XXV.

Rats

Sica and Cerecedo (1948) hold the view that there is no dietary factor necessary to the rat for lactation only; a diet which is qualitatively adequate for reproduction will in their opinion be adequate also for lactation. This conclusion is based on an analysis of the relation between birthweight and survival of rats born to mothers on a stock diet, the purified basal diet described on page 77 and on the basal diet variously supplemented with folic acid, biotin, xanthopterin and milk. Litters were reduced to 6 on the third day of lactation. The average birthweight of the young in litters of which no young survived to weaning was 4·9 g.; 47·4 per cent. were below 5·0 g. and only 0·5 per cent. over 5·4 g. Of the young born in litters which survived, the average birthweight was 5·7 g.; only 4·1 per cent. weighed less than 5·0 g. and 74·3 per cent. weighed over 5·4 g.

TABLE

DEGREE OF SUCCESS IN REARING OF YOUNG ATTAINED

Author	Animal	Basal Diet, Percentage				Fat-soluble Vitamins			
		Protein	Carbohydrate	Fat	Salts	A	D	E	
(1) Jukes (1940)	Black rats	Casein 25	Sucrose 60	Crisco 8	5	+	+	Maize oil	—
(2) Unna *et al.* (1941)	,, ,,	,, 18	,, 68	,, 8	4	+	+	—	—
(3) Henderson *et al.* (1942)	Black or piebald rats	,, 18	,, 73	Maize oil 4	4	+	+	α-Tocopherol	—
(4) Vinson and Cerecedo (1944)	Rats	,, 30	α-Cellulose 2 Sucrose 48	Crisco 10	5	+	+	α-Tocopherol	— —
(5) Richardson and Hogan (1945)	Rats	"Purified Diet"				+	+	α-Tocopherol	+
,, ,, ,, ,,	,,	,,				,,	,,	,,	,,
,, ,, ,, ,,	,,	,,				,,	,,	,,	,,
,, ,, ,, ,,	,,	,,				,,	,,	,,	,,
(6) Ershoff (1943); Ershoff and McWilliams (1943)	Albino rats	Casein 22 +0·3 cystine	Sucrose 73·2	—	4·5	—	—	—	—
,,	,, ,,	,,	,,	—	,,	—	—	—	—
(7) Ershoff (1946a)	Albino rats Hooded rats	Casein 22	Sucrose 73·5	Maize oil 500 mg. daily	4·5	+	+	α-Tocopherol	+
(8) Nelson *et al.* (1946)	Rats, Long-Evans strain	,, 24	,, 64	Crisco 8	4	+	+	Maize oil	—
(9) Nelson and Evans (1947c)	Rats, Long-Evans strain	,, 24	,, 64	,, 8	4	+	+	,,	+
	,, ,, ,, ,,	,,	,,	,,	,,	,,	,,	,,	,,
(10) Folley *et al.* (1947)	Rats, Black and white	,, 20 +0·2 cystine	,, 60	Lard 3 Groundnut oil 12	5	+	+	α-Tocopherol	+
,, ,, ,, ,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
(11) Foster *et al.* (1943)	Mice	Fibrin 25	Regenerated cellulose 4 Glucose 67	Linoleic acid	4	+	+	α-Tocopherol	+
(12) Cerecedo and Vinson (1944a)	Mice	Casein 30	α-Cellulose 2 Sucrose 48	Crisco 10 Lard 5	5	+	+	α-Tocopherol	—
,, ,, ,, ,,	,,	,,	,,	,,	,,	,,	,,	,,	—

XXV

BY FEMALE RATS AND MICE ON PURIFIED DIETS

B Complex Vitamins (mg. per 100 g. Ration)										Rearing of Young
	B_1	Riboflavin	B_6	Nicotinic Acid	Choline	Ca Pantothenate	Inositol	p-Amino-benzoic Acid	Folic Acid	Biotin	
(1)	0·2	0·5	0·2	1·0	50	2·8	—	—	—	—	Young not reared
(2)	0·8	0·8	0·8	10·0	100	0·1	—	—	—	—	3 generations successfully reared
(3)	0·2	0·3	0·2	0·25	100	2·0	—	—	—	—	4 generations reared. Some does unsuccessful in rearing young
(4)	2·0	2·0	2·0	—	50	4·0	—	—	—	—	Weaning performance inferior to that of stock rats; does lost weight
	,,	,,	,,	—	,,	,,	—	—	+	—	Loss of weight prevented
(5)	1·0	1·0	1·0	5	200	2·0	100	50	—	20	69·7 per cent. of young weaned
	,,	,,	,,	,,	,,	,,	omitted	,,	—	,,	44·3 ,, ,, ,,
	,,	,,	,,	omitted	,,	,,	100	,,	—	,,	60·4 ,, ,, ,,
	,,	,,	,,	5	,,	,,	,,	omitted	—	,,	75·8 ,, ,, ,,
(6)	0·5	1·0	0·5	10	100	10	40	15	—	—	No doe nursed her young
	,,	,,	,,	,,	,,	,,	omitted	11	—	—	3 out of 8 litters reared
(7)	0·2 or 20·0	0·4 or 40·0	0·2 or 2·0	—	120	3·0 or 20·0	—	—	—	—	89 per cent. of young reared (albino)
									—	—	No young reared (hooded)
(8)	0·5	1·0	0·5	2·0	100	5·0	40	1·0	—	—	90 per cent. of young reared Leucopenia in does
(9)	0·5	1·0	0·5	2·0	100	5·0	40	1·0	—	—	95 per cent. of young reared. See Table XXIII for leucocyte and granulocyte count
		,,	,,	,,	,,	,,	,,	,,	0·11 (Synthetic pteroyl-glutamic acid)	—	,, ,, ,,
(10)	—	+	+	+	+	+	+	+	—	—	50 per cent. of young reared
	,,	,,	,,	,,	,,	,,	,,	,,	+	+	50 ,, ,, ,,
(11)	1·0	1·0	1·0	6·0	60	6·0	6·0	15·0	—	—	4 generations reared; 65 per cent. of young weaned
(12)	2·0	2·0	2·0	—	50	4·0	—	—	—	—	40 per cent. of young reared
	,,	,,	,,	—	,,	,,	—	—	+	—	80 ,, ,, ,,

Of 46 litters in which young failed to survive, 29 litters failed to survive the third day of lactation. Sica and Cerecedo believed the causes of such failures could be traced to the reproduction process. Of 27 litters cast on the stock diet 4 failed to survive. Birthweights of these young fell in and below the critical range of 5·0 to 5·4 g. On the unsupplemented purified diet 7 of 10 litters cast failed to survive. The young in only 1 of these litters had birthweights above the critical range.

Mice

Mirone *et al.* (1948) analysed the mortality records of 2005 litters of mice from does on purified diets and found that 91 per cent. of litter deaths occurred during the first 4 days after birth. Of 127 mothers whose litters died during the first 4 days, 57 were able to rear foster litters born to females on stock or good experimental diets. On the basis of this evidence Mirone *et al.* subscribe to the view of their colleagues based on work with rats (Sica and Cerecedo, 1948) that inability of does reared in their laboratory on certain purified diets to raise their young successfully to weaning was due to inadequacy of the diets for the production of viable young rather than inadequacy for lactation. Mirone *et al.* suggest that a more reliable picture of a diet may be gained by expressing performance in terms of the following indices: gestation index, number of litters born alive: number of pregnant animals; viability index, number of litters alive on 4th day: number of litters born alive; over-all reproduction index, number of litters alive on 4th day: number of pregnant animals; lactation index, number of litters weaned: number of litters alive on 4th day.*

Syrian hamsters

Hamilton and Hogan (1944) found that hamsters reared from weaning on a synthetic diet of purified casein 20, cerelose (glucose) 65, lard 7, cellulose 3, salt mixture 4 and vitamin A and D concentrate 1 per cent., supplemented in mg. per 100 g. basal diet, by vitamin K 3·0, α-tocopherol 2·5, vitamin B_1 0·8, riboflavin 1·6, vitamin B_6 1·2, Ca pantothenate 1·0, nicotinic acid 5·0, choline chloride 400, inositol 250, and *p*-aminobenzoic acid 100, grew well and reached full maturity, and females produced at least 1 litter. Few produced more than 1 litter, while females on a stock diet produced up to 5 successive litters, and it was concluded that the synthetic diet was deficient in some unidentified vitamin. The results of adding biotin were inconclusive.

Dogs

Morris *et al.* (1945) reported that certain rations which were adequate for adult dogs and permitted the production of apparently normal young by pregnant females were inadequate for successful rearing of the young. The pups showed anaemia as well as a retarded rate of weight increase. Subcutaneous administration of liver extract to suckling pups resulted in an increase in Hb and red cell counts and improved clinical appearance, while untreated pups suckling the same mother died. Administration of liver extract subcutaneously to the bitch during gestation and lactation resulted in the rearing of healthy pups. Tests with *Lactobacillus casei* factor of liver extract and with vitamin B_6 were made to determine the factor or factors in the liver extract which were lacking in the mother's diet. The detailed results are to be reported later.

SUMMARY

The prerequisite of all experimental work with laboratory animals is a stock diet upon which the stock colony will thrive and reproduce itself efficiently. An imposing array of stock diets which permit satisfactory growth of young animals could be assembled from the literature

* Mirone *et al.* include as litters alive those litters in which young survive regardless of the number surviving. When they say that 91 per cent. of litter deaths occurred in the first 4 days, they refer to litters in which all the young died. (Private communication, 1948).

and descriptions of some will be found in publications on animal colony maintenance (Loosli, 1945) and the care and management of laboratory animals (Worden, 1947). Much less information is available about reproductive performance on stock diets. A few stock rations for which records of reproduction are available have been presented at the beginning of this communication. These data rightly precede an account of attempts to evaluate the requirements of animals for individual nutrients since invariably the diets have been designed as the result of trial and error and in few instances have their detailed composition in terms of nutrients been determined. Slanetz (1943) has contributed detailed analyses of 6 selected stock rations used in scientific institutions in the United States, for rats, mice, rabbits and guineapigs but unfortunately records of the reproductive performance of animals on these diets are not available.

The basis of stock diets is usually one or more cereal and of synthetic diets a pure carbohydrate with addition of protein and such other substances, in relatively small quantities, as may be considered necessary. When, in what follows, requirements are quoted as per 100 g. diet or food, this type of diet is to be understood.

The food consumption of rats on stock diets during the reproductive cycle has been measured and the energy requirements of the doe for the production and rearing of her litter have been calculated. From the limited data available it would appear that in litters of rats averaging 7 young, in all about 80 Cal. are required over maintenance for each pup during the gestation period of 22 days and about $3\frac{1}{2}$ times that amount for each pup during the 22 days of lactation. Temporary restriction of the quantity of food consumed delays sexual maturation in laboratory animals but does not impair the fertility of animals subsequently fed to appetite.

The ideal approach to the problem of evaluating the needs of laboratory animals for specific nutrients would be to begin with a diet of natural foods of known chemical composition, adequate for satisfactory growth, reproduction and rearing of young, and reduce the level of one nutrient at a time to the point where the diet ceases to be satisfactory. It is difficult, if not impossible, to remove one nutrient and one only from a mixed diet. For instance, reduction of meat to reduce the protein content or reduction of milk to reduce the Ca content reduces the level of other nutrients which may be of importance. It is natural, therefore, that investigators have tried to build up artificial diets in which nutrients are supplied singly. In such diets the carbohydrate component is usually a pure sugar or starch which can be replaced by purified casein so that the protein content is increased without loss of other essential nutrients. Single nutrients such as $CaCO_3$ and synthetic vitamins are added to the basal diet until no further improvement in reproductive performance is obtained. This technique could be extended to include individual amino-acids in place of casein. If a purified diet could be built up which compared favourably with any known combination of natural foods it might be possible by progressive reduction in the level of one constituent at a time, to the point where reproductive performance was impaired, to express the requirements of the animal for each nutrient in relation to a standard diet of known chemical composition. To express requirements absolutely without relation to the levels of other nutrients would be to ignore the increasing weight of evidence, recently reviewed by Moore (1945) and Ershoff (1948), that the dietary requirement of almost every nutrient depends to some extent on the amount of some other nutrient or nutrients in the diet. For instance, the requirement of choline depends on the amount of methionine present in the diet and might therefore be different for animals on diets containing 20 per cent. of meat protein and 20 per cent. of casein which is low in methionine. The establishment of requirements from the results of experiments with purified diets leaves out of account possible differences in the effect of

natural and purified foods on the intestinal synthesis of certain essential nutrients. It is also obvious that, with so many individual constituents to test at different levels, it will be a long time before all the reasonable combinations can be tried and that some of the results reported below may be valid only for the assortment of items tested.

It is disappointing, but not perhaps surprising, that a study of the available literature shows that little is known exactly of the requirements of laboratory animals for individual nutrients consumed either in the form of natural or purified foods. With these reservations in mind about the relationships between nutrients the evidence may be summarised briefly.

Protein

Diets low in protein or lacking in certain essential amino-acids do not permit normal sexual development in laboratory animals. A level of 10 per cent. of mixed protein permits normal sexual rhythm in the female rat; 20 per cent. of mixed protein, 25 to 30 per cent. of casein or 20 per cent. of casein supplemented with 0·2 per cent. of cystine is adequate for reproduction and the rearing of young rats. When the diet of rats contains 40 to 50 per cent. of protein, the efficiency of utilisation of protein is probably impaired. Very high levels appear to have an adverse effect on the sexual development of the female rat.

Minerals

For rats, 0·22 per cent. of Ca in the diet appears to be the critical level necessary to allow growth and reproduction over several generations but the optimum lies between 0·3 and 0·8 per cent., with a Ca : P ratio of between 1·0 and 2·0. Retarded sexual development in rats on diets low in P has been reported but paired feeding experiments indicate that inanition was the cause. Vitamin D appears to have a beneficial effect when added to diets low in Ca and as a supplement to a rachitogenic diet it prevents foetal rickets.

A level of 50 mg. Mg per 100 g. in the diet of rats appears to be adequate.

Excellent results have been obtained with rats on a stock diet containing 33 μg. I per 100 g.

For the rat 0·05 mg. Cu daily is probably adequate during the reproductive period.

Evidence has been presented that deficiency of Mn in the diet interferes with the normal oestrous cycle in mice and causes ovarian and testicular degeneration in rabbits. The conflicting results obtained with rats have been discussed (page 40). The addition of 1 mg. or more of Mn per rat daily to a synthetic diet for rats seems to be necessary if viable young are to be produced; a diet of natural foods with less Mn may be adequate.

In rats sexual maturity was delayed and the oestrous cycle disturbed on a diet containing 0·002 per cent. Na. A good stock diet has been reported to contain 0·4 per cent. Na on a dry matter basis.

A level of 0·14 to 0·18 per cent. of K in the diet of rats seems to be necessary for normal sexual development, reproduction and the rearing of young. The changes in the sex organs in rats on K-free diets appear to be due to inanition.

Beneficial effects of supplementing diets with Al, B or F have not been demonstrated.

The toxic effects of excess of F and Se are well known.

Essential Fatty Acids and Fat

The presence of linoleic acid or other unsaturated fatty acid is essential in the diet of rats for normal oestrous cycles, prevention of testicular degeneration and for normal

reproduction. The requirement is probably influenced by the intake of many other substances such as vitamin B_6 and of α-tocopherol. Opinion is divided as to the need for fat *per se* for the successful rearing of young.

Fat-Soluble Vitamins

Cornification of the vaginal epithelium in rats is the classical sign of vitamin A deficiency. Diets low in vitamin A cause testicular degeneration in rats and mice and may adversely affect the oestrous cycle. Disturbance of the oestrous cycle appears to be due to inanition rather than to lack of vitamin A *per se*. Female rats on diets low in vitamin A have difficulty in giving birth to live young and the dead are usually characteristically malformed. A level of 1 p.p.m. of carotene in the diet or 7 μg. daily appears to be adequate for reproduction and 5 p.p.m. for the successful rearing of young. Reproduction in guineapigs is affected by lack of vitamin A but their requirements are not known.

The resorption of embryos in female rats, mice and guineapigs maintained on diets deficient in vitamin E and muscular dystrophy in the young of females deprived of vitamin E during gestation are well attested phenomena. Male rats deprived of vitamin E in early life show testicular degeneration but in mice levels of vitamin E low enough to cause resorption gestations in females do not cause testicular degeneration in the male. Rabbits can show severe muscular dystrophy in the absence of testicular damage. Very different estimates have been made of the optimum level of vitamin E in the diet of rats for successful reproduction; possible reasons for the discrepancies have been discussed (page 60).

In rabbits and guineapigs on diets deficient in vitamin K, abortions, which can be prevented by addition of vitamin K to the diet, have been described. The existence of an unidentified "rabbit ovulating factor" or factors in grass juice has been postulated. It is generally agreed that the rat synthesises sufficient vitamin K in the intestine to obviate the need for inclusion of this vitamin in purified diets but some workers have suggested, on rather slender evidence, that this may not be true for fat-free diets.

Vitamin C

Although the guineapig's need of vitamin C for the prevention of scurvy is well known, the exact vitamin C requirement for normal reproduction and the rearing of young has not been determined. Changes in the ovaries and the occurrence of abortion or resorption of embryos on vitamin C-free diets have been reported but not the effect of adding vitamin C to the deficient diets. The suggestion that the pregnant guineapig or its foetus synthesises vitamin C has been debated and is still in doubt.

Vitamin B Complex and Choline

Lack of vitamin B_1 upsets the sexual rhythm of female rats, probably because of the accompanying inanition. The minimum amount necessary for normal reproduction and the rearing of young is not known, but many generations have been reared successfully on a stock diet containing 400 μg. per 100 g. of food.

Lack of riboflavin also upsets the oestrous cycle in female rats. On a riboflavin deficient diet, if allowed to mate before anoestrus becomes permanent, they produce young of which a high percentage show congenital abnormality. The requirement of the rat for reproduction and the rearing of normal young appears to lie between 300 and 1000 μg. per 100 g. of food.

Lack of vitamin B_6 in the diet causes sterility in male rats. In female rats deficiency of vitamin B_6 in the diet from parturition results in a convulsive syndrome in the suckling

young, apparently due, not to any inherent defect in the young nor to any interference with milk secretion, but to a lack of vitamin B_6 in the milk. The requirement for vitamin B_6 is not known but 200 μg. per 100 g. of diet appears to be ample.

There is evidence that pantothenic acid is essential in the diet of pregnant female rats for the successful production and rearing of young. The requirement for reproduction appears to be between 2·8 and 5·0 mg. per 100 g. of diet.

There is evidence that under some experimental conditions female rats and mice maintained on purified diets with a supplement of choline are better able to rear their young than when choline is omitted from the diet. In other instances no beneficial effect has been demonstrated. It is probable that the results obtained with choline supplements depend upon the strain of animal used, and upon such variables as the supply of cystine or of substances such as methionine capable of contributing methyl groups for the synthesis of choline *in vivo*. An adverse effect of omission of choline from a purified diet for Syrian hamsters has been reported; fewer young were reared than when choline was included.

Inclusion of nicotinic acid, inositol and *p*-aminobenzoic acid in purified diets is apparently unnecessary for successful reproduction in rats and mice. Some investigators have found that inclusion of these vitamins in the diet of female rats results in an improvement in the percentage of young reared to weaning. Others have reported no improvement and indeed, with inositol and *p*-aminobenzoic acid, adverse effects have been described. Omission of inositol from a purified diet for Syrian hamsters has been reported to impair the production of live young and omission of nicotinic acid from the same diet reduced the number of young hamsters reared.

Addition of folic acid or synthetic pteroylglutamic acid to a purified diet containing all of the above B vitamins except biotin has been shown to exert a favourable influence on the ability of young female rats and mice to gain weight during lactation. It has been shown also that the leucocyte count and percentage of granulocytes in maternal rat blood at the weaning of litters of the Long-Evans strain approaches more nearly to the normal for stock rats when pteroylgutamic acid is included in the purified diet at a level of 110 μg. per 100 g. of diet.

The inclusion of biotin in purified diets for rats does not appear to be necessary for the successful reproduction and rearing of young unless the diet contains avidin. In mice a beneficial effect from addition of biotin to a purified diet has been reported.

The existence of an unidentified substance or substances essential for optimum lactation of female rats and mice has been postulated; but 95 per cent. of young rats and 80 per cent. of young mice have been reported as being reared by females on purified diets containing only the known essentials.

There has been a tendency to class as essential for lactation supplements which, when added to purified diets from the beginning of gestation or earlier, raise the percentage of liveborn young reared to weaning. It was suggested in the introduction that failure to rear young may mean that the young were not viable at birth because of faulty nutrition *in utero*. Now, Mirone, Cerecedo and others have demonstrated with purified diets supplemented with vitamins of the B complex that most of the litter deaths of mice occurred during the first 4 days of life and that about 50 per cent. of mothers whose litters died during this period were able to rear healthy foster-litters. They suggest that a more reliable index of lactation would be the percentage of litters reared of litters alive at 4 days. The choice of the 4-day limit to distinguish between deaths due to prenatal and those due to postnatal causes may be open to question and the calculation of the index in terms of litters may raise difficulties but the procedure seems to be an advance on the older method.

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